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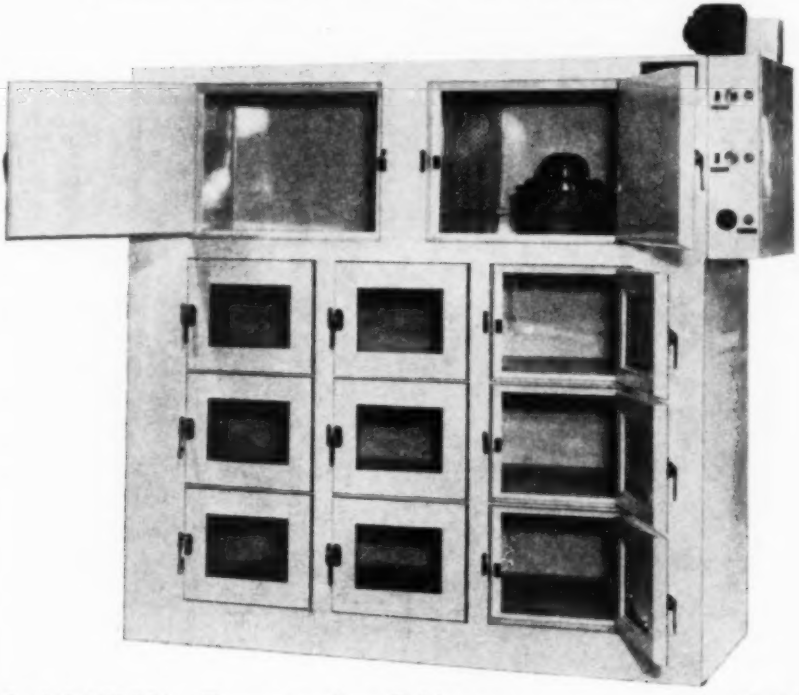

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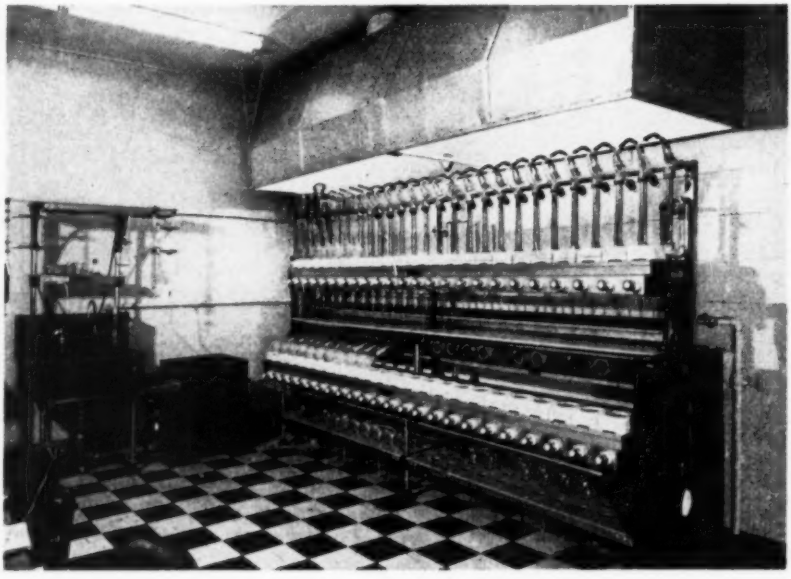

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CEREAL CHEMISTRY

VOL. XXIX JANUARY, 1952 NO. 1

EFFECT OF OXIDIZING AGENTS UPON DOUGH EXTENSOGRAMS [1]

DONALD E. SMITH and JOHN S. ANDREWS

ABSTRACT

The Extensograph has been used to measure the influence of various treatments on dough characteristics. The atmosphere in which the doughs are mixed has a profound effect. Doughs mixed in nitrogen and subjected to the dough handling schedule used in baking are soft, very pliable, and can be stretched to relatively long strands with slight forces. Doughs mixed in oxygen and handled similarly require much greater forces to stretch them and breaking occurs at much shorter distances.

The extent of these differences depends on the handling treatment given the doughs. The influence of atmosphere is not immediate but is revealed with the lapse of time; a reworking of the dough is necessary to bring out the effect. Doughs which are mixed in nitrogen or oxygen are quite similar in Extensograph properties when these are measured immediately after mixing. Both are intermediate in extensibility and resistance to extension. If the doughs are not handled between mixing and measurement, they slacken and become more extensible. On the other hand, if they are allowed to stand and are then molded or "saddled" and tested at a standard time interval after molding, the dough mixed in oxygen is elastic and slightly extensible, whereas the dough mixed in nitrogen is slack and very extensible.

The addition of maturing agents, increased mixing, and increased fermentation all tend to increase elasticity and decrease extensibility of doughs. These effects are also related to the handling treatment that the dough receives. The mixing effect is related to the incorporation of air, but small effects also are shown with vacuum- or nitrogen-mixed doughs.

In flour quality testing there is a need for techniques that will record dough characteristics observed by a skilled baker and to relate these characteristics to flour quality. The Extensograph measures extensibility and resistance to extension of doughs and may be used in a variety of ways.

Munz and Brabender (4, 5), and Merritt and Bailey (3) have measured dough physical properties with the Extensograph. They showed

[1] Manuscript received July 10, 1951. Presented at the Annual Meeting May, 1951. Paper No. 113, Journal Series, Research Laboratories, General Mills, Inc.

that if doughs were molded directly from the mixer and tested at intervals, the curve height (resistance to extension) decreased and the curve length (extensibility) increased with time after mixing. The dough became more extensible with rest. If, however, the doughs were not molded immediately but were molded at various intervals of time after mixing, the extensibility decreased with the rest time and the curve height increased. Such doughs became less extensible with time.

Fisher, Aitken and Anderson (2) determined the effects of initial mixing procedures upon extensograms. With the mild mixing action of the Hobart mixer, little change in curve height or curve length occurred as the mixing time was increased and there was little difference between 45-minute and 135-minute extensograms. With severe mixing action (Swanson mixer), extensibility decreased and resistance increased with increasing mixing time, and these changes were greater in 135- than in 45-minute extensograms.

In the course of some investigations on the factors which influence the baking character of flours, the Extensograph was employed to evaluate the effect of different flour oxidation treatments. These results will be reported here.

Materials and Methods

Untreated patent and first clear flours milled from a Montana winter wheat were used in this work. Unleavened doughs containing 2% salt and sufficient water to give a maximum Farinograph consistency of 500 Brabender units when 480 g. of the dough were mixed in air were employed.

Mixing was done in the Brabender Farinograph, the Hobart mixer with a McDuffee bowl and the Swanson mixer as indicated later. The Extensograph setting was such that a 100 g. weight added to the saddle containing the dough gave a reading of 60 units.

All doughs were held at 30°C. during the experiments. When doughs were mixed in the Farinograph in nitrogen or oxygen, the gas at atmospheric pressure was passed into the dough during mixing through a hole in a cover plate. The gas was passed into the flour for 10 minutes before the dough was mixed to flush out air.

Vacuum mixing was done with a Hobart mixer placed inside a large metal chamber that could be evacuated. A vacuum of 26 in. Hg was used during mixing, following which it was released with nitrogen. This same apparatus was employed for mixing doughs in nitrogen. A vacuum was drawn on the Hobart mixer containing the dough ingredients. This was released with nitrogen and the nitrogen pressure increased to 15 in. Hg.

Results

Effect of Mixing in Oxygen and Nitrogen. In the first experiments a dough handling technique similar to that used in baking was used. Unleavened doughs containing 2% salt (flour basis) were mixed in the Farinograph for 10 minutes. The doughs were scaled to 150 g. portions and submitted to the following schedule (hours).

Mix	*1 Punch*	*2 Punch*	*Saddle*	*Draw Curve*
0	1.75	2.5	3	4

Doughs were punched by hand at the indicated times. At saddle time they were folded five times and passed through the Extensograph molder. (Gentle preliminary rounding by hand facilitated this molding operation. The Extensograph rounder was not employed since the later-used leavened doughs tended to stick to the plate.) The curve was drawn after one hour in the saddle.

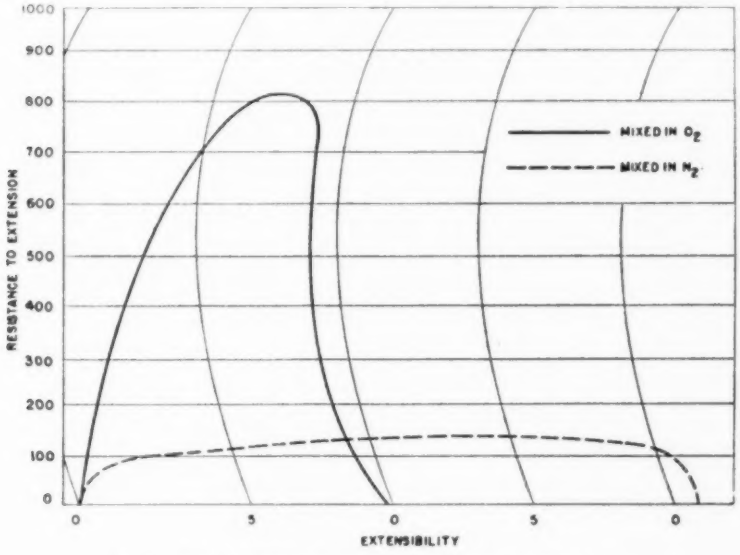

FIG. 1. Effect of atmosphere upon the extensograms of doughs placed in the saddle three hours after mixing.

With this technique curves as given in Fig. 1 were obtained with first clear doughs mixed in nitrogen and oxygen atmospheres. The resistance to extension of the oxygen-mixed dough is much greater and the extensibility is much less than that of the nitrogen-mixed dough.

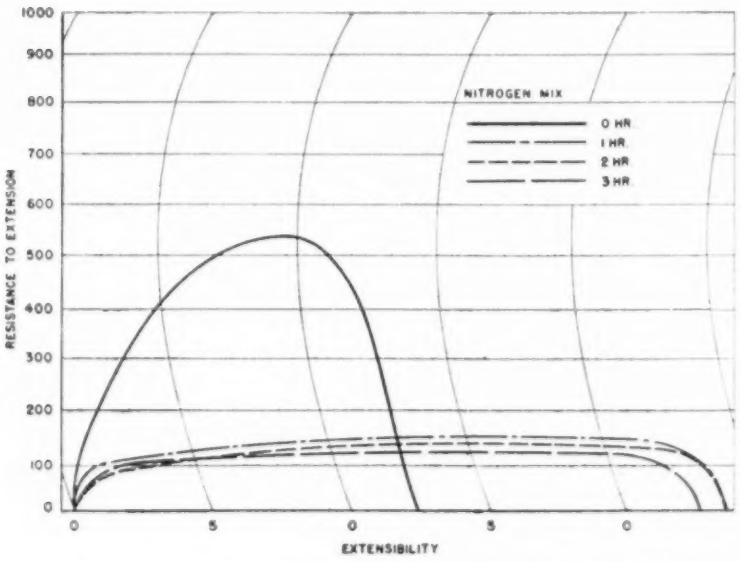

FIG. 2. Effect of time after saddling upon the extensograms of doughs mixed in nitrogen and saddled immediately after mixing.

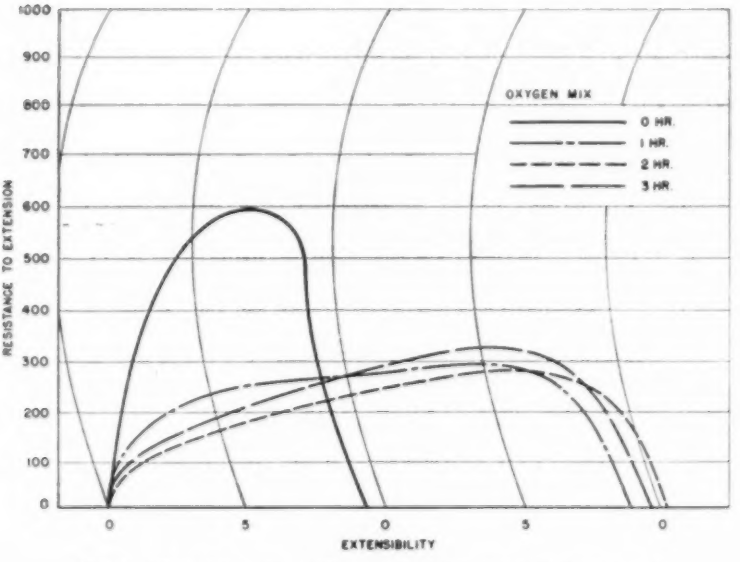

FIG. 3. Effect of time after saddling upon the extensograms of doughs mixed in oxygen and saddled immediately after mixing.

Effect of Time and Dough Handling. To secure information about the rate of the action of oxygen, Montana winter wheat first clear doughs were mixed 10 minutes in the Farinograph and placed in the Extensograph saddle directly from the mixer. Curves were drawn after the times given with the results shown in Figs. 2 and 3 for nitrogen- and oxygen-mixed doughs, respectively.

The doughs mixed in nitrogen or oxygen gave initial high curves and slackened with time. The oxygen-mixed dough did not become as extensible or lose its resistance to extension as much as that mixed in nitrogen.

What causes this apparent softening in the resting doughs? It cannot be attributed to a breakdown of the gluten because when these

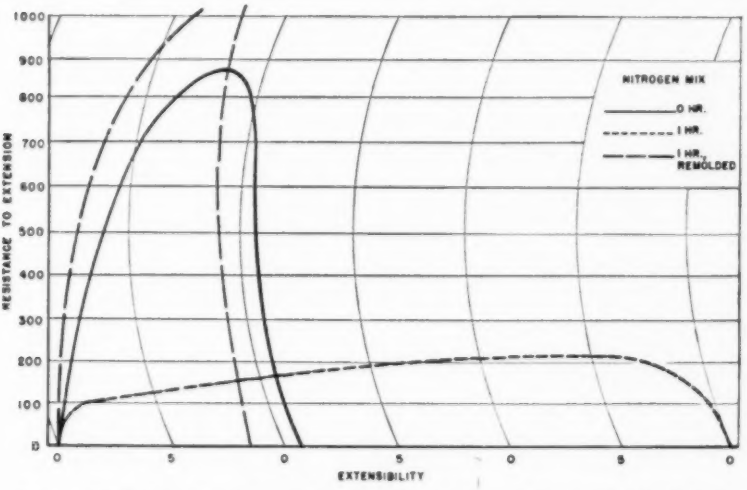

FIG. 4. Effect of rest time and remolding upon the extensograms of doughs mixed in nitrogen.

same slack doughs were remolded and tested immediately, the curves went off the scale. This is shown in Figs. 4 and 5 with doughs mixed in nitrogen and oxygen, respectively. The solid line was obtained with a dough tested immediately after mixing. When this dough was allowed to rest one hour, the curve shown by the lower dotted line was obtained. When, however, this last dough was remolded and tested immediately, the curve was again high, even higher than from the dough tested directly after mixing. This illustrates the extreme importance of considering the history of the dough in evaluating changes in dough characteristics.

In another experiment a dough was mixed in nitrogen and another in oxygen, but the doughs were not saddled until the times given in

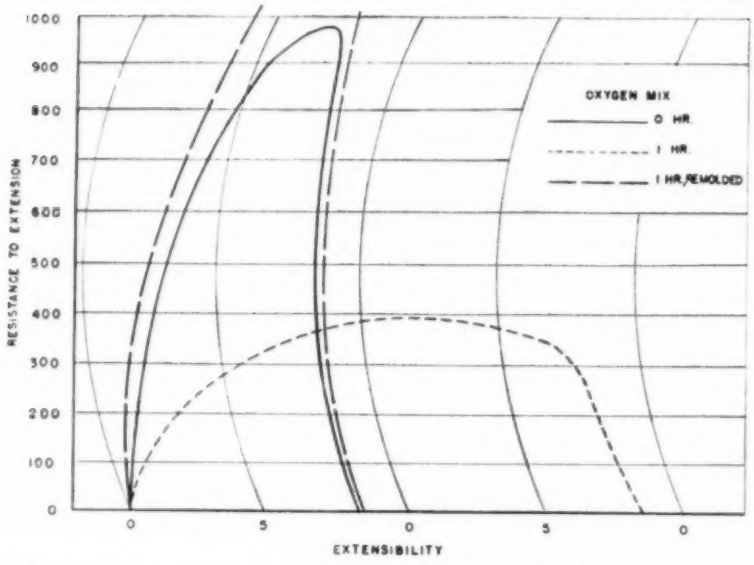

FIG. 5. Effect of rest time and remolding upon the extensograms of doughs mixed in oxygen.

Figs. 6 and 7. All doughs were held in the saddle one hour before the curves were drawn. Doughs were not handled after mixing until they were placed in the saddle.

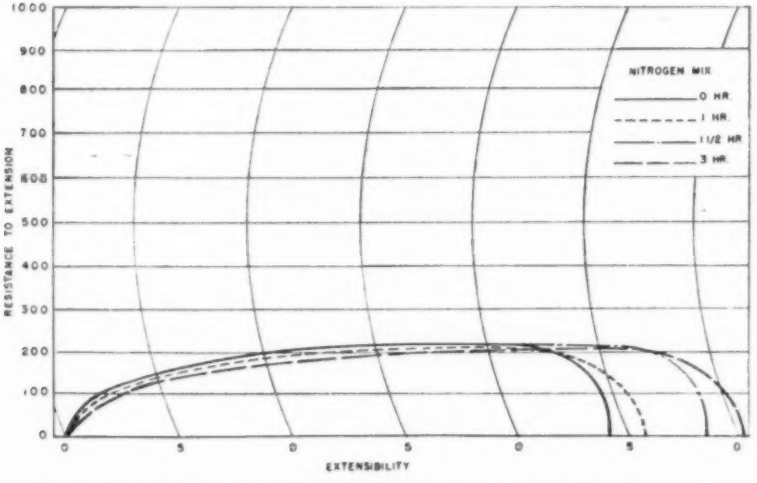

FIG. 6. Effect of time before saddling upon the extensograms of doughs mixed in nitrogen.

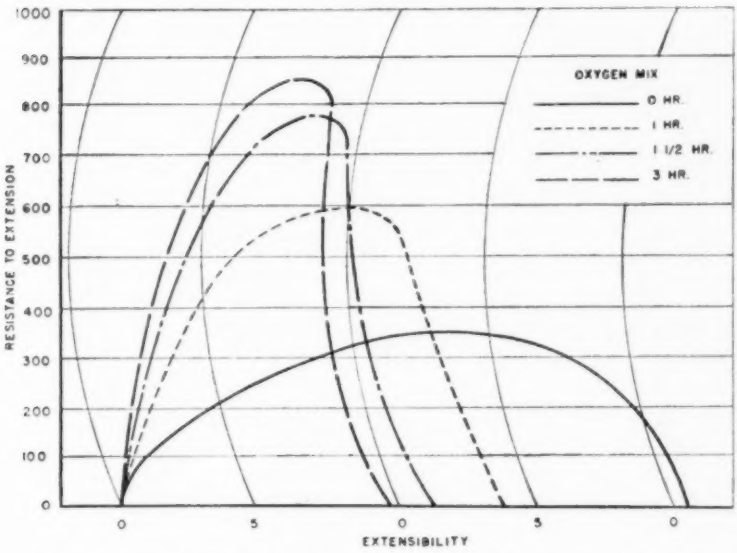

FIG. 7. Effect of time before saddling upon the extensograms of doughs mixed in oxygen.

The marked effect of oxygen upon the physical properties of a flour dough is shown with respect to time. The dough structure must also be changed by molding after the time interval to give the effect. Doughs mixed in nitrogen did not change much with time even though they were held and molded in air after mixing. The influence of rest-time and manipulation on the physical properties of the oxygen-mixed doughs is illustrated by the following data.

Saddle hr.	*Draw Curve* hr.	*Curve Height* B.U.
0	0	615
0	1	310
0	3	325
3	4	855

The results in Table I show that time and manipulation are also required to reveal the effect of oxidation (oxygen) upon leavened doughs. These doughs were made in the Farinograph from the first clear flour using a commercial straight dough formula. The nitrogen-mixed doughs showed some increase in curve height when a reaction time was given previous to saddling. Unleavened doughs mixed in nitrogen did not increase in curve height with a reaction time before being placed in the saddle, but the leavened doughs showed an increase. Some of the effects of oxidation are associated with fermentation.

TABLE I

EFFECT OF TIME AND MANIPULATION UPON THE PROPERTIES OF LEAVENED DOUGHS MIXED IN NITROGEN AND IN OXYGEN

Atmosphere	1 P	Saddle	Draw Curve	Height	Length
	hr.	*hr.*	*hr.*	*B.U.*	*B.U.*
Nitrogen		0	2	170	12
Nitrogen		1	2	240	16
Nitrogen	1	2	3	285	16
Oxygen		0	2	280	22
Oxygen		1	2	690	15
Oxygen	1	2	3	700	12

The effects of time and manipulation on the physical properties of unleavened salted doughs subjected to a regular baking schedule are shown in Fig. 8. The data for these curves were obtained by testing ten separate 150 g. portions of dough, one at each of the points indicated.

All doughs gave appreciable heights when curves were drawn immediately after mixing and they all slackened to the next point of physical manipulation. At the first punch, those mixed in air or

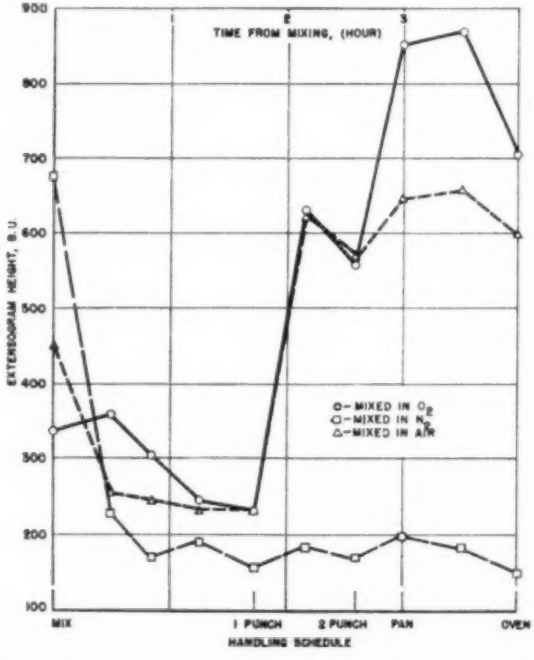

FIG. 8. Effect of handling and mixing atmosphere upon the extensogram height of doughs.

oxygen showed a marked increase in curve height whereas those mixed in nitrogen showed little change. The former slackened until the second punch when both increased again, but the oxygen-mixed dough increased more than the air-mixed dough. Another rise was given after panning-time and a slight slackening at oven-time. At oven-time there was a great difference in the character of the oxidized and unoxidized doughs.

TABLE II

EFFECT OF TIME AND HANDLING UPON THE ACTION OF DOUGH IMPROVERS

1 P	2 P	3 P	Saddle	Draw Curve	Height	Length
hr.	*hr.*	*hr.*	*hr.*	*hr.*	*B.U.*	*B.U.*
167 p.p.m. Bromate						
			0	1	415	20
			0	3	415	22
			0	5	410	22
			0	1	320	20
1.5			2	3	1,175	11
1.75	2.75	3.5	4	5	1,100	9
25 p.p.m. Chlorite						
			0	1	305	20
			0	3	265	21
			0	5	265	20
1.5			2	3	610	15
1.75	2.75	3.5	4	5	680	16
22 p.p.m. Chlorine Dioxide						
			0	1	720	14
			0	3	670	16
			0	5	570	17
1.5			2	3	1,100	9
1.75	2.75	3.5	4	5	1,000	9

Effects with Dough Improvers. The effect of time and dough handling upon the action of potassium bromate, sodium chlorite and chlorine dioxide on unleavened, salted first clear flour doughs is shown in Table II. These doughs were mixed in the Farinograph for 10 minutes in a nitrogen atmosphere to eliminate the effects of air.

All of these improvers, particularly the chlorine dioxide at the level used, had some effect upon the doughs that were saddled immediately. Comparable doughs mixed in nitrogen without the addition of oxidizing improvers gave curve heights of about 200 B.U. The full effect of the

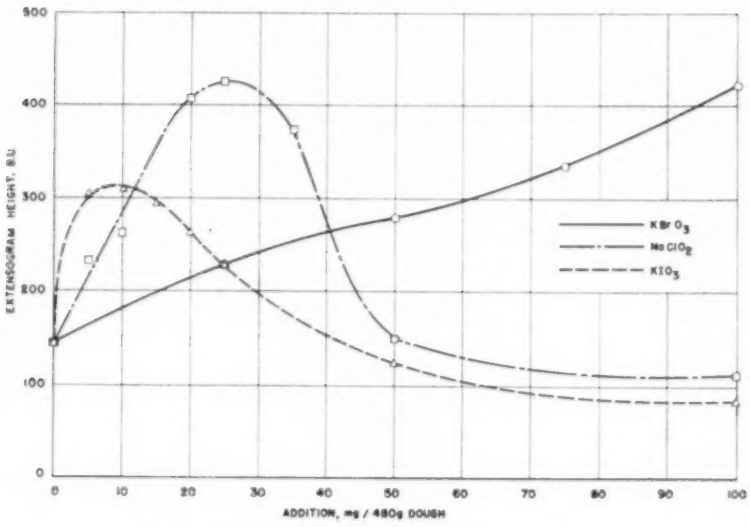

FIG. 9. Effect of dough improvers upon the extensogram height of no time doughs mixed in nitrogen.

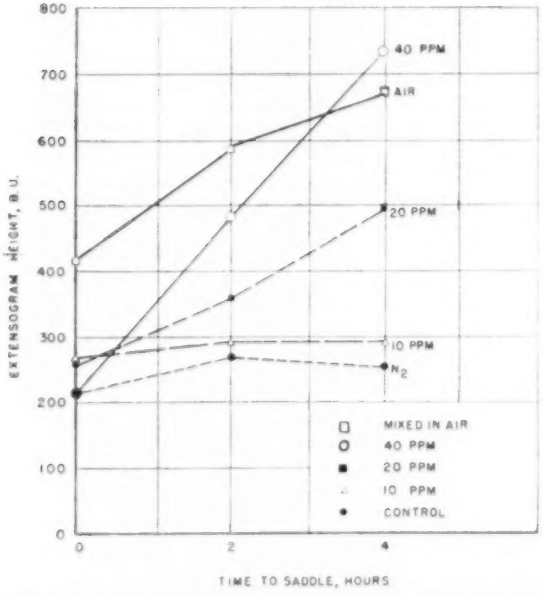

FIG. 10. Effect of potassium bromate upon the extensogram height of unleavened doughs mixed in nitrogen.

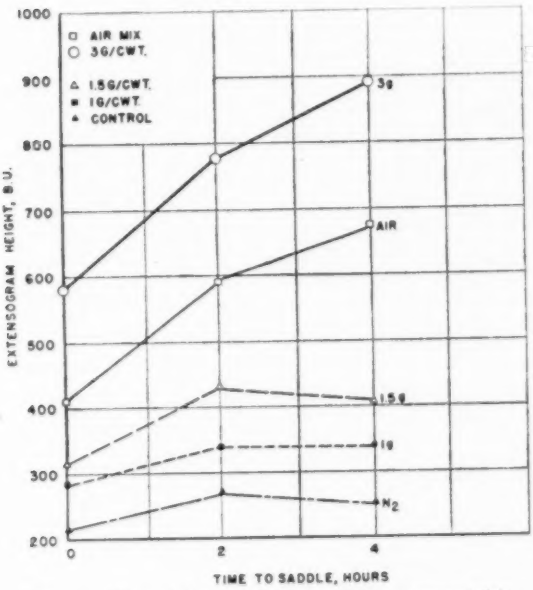

FIG. 11. Effect of chlorine dioxide upon the extensogram height of unleavened doughs mixed in nitrogen.

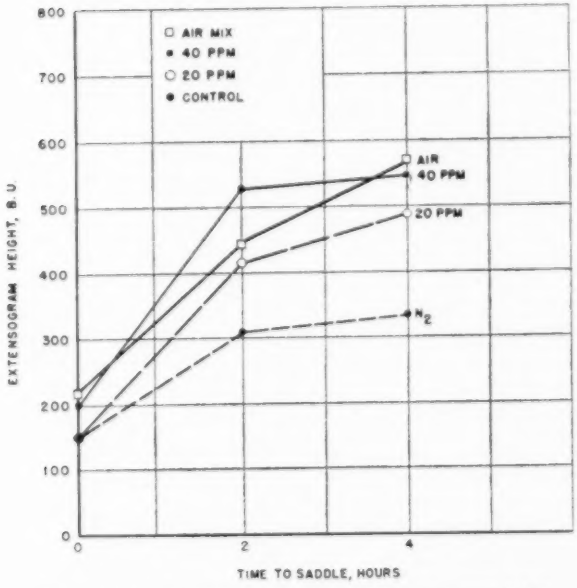

FIG. 12. Effect of potassium bromate upon the extensogram height of leavened doughs mixed in nitrogen.

improvers was not given until a reworking occurred after a reaction time.

Mixing doughs in nitrogen enables one to study the effect of oxidizing agents exclusive of air oxidation. Figure 9 shows this effect as obtained with three oxidizing agents added to unleavened first clear flour doughs mixed 10 minutes in the Farinograph, saddled directly from the mixer and tested after one hour in the saddle. Both potassium iodate and sodium chlorite were much more effective than potassium bromate under these conditions.

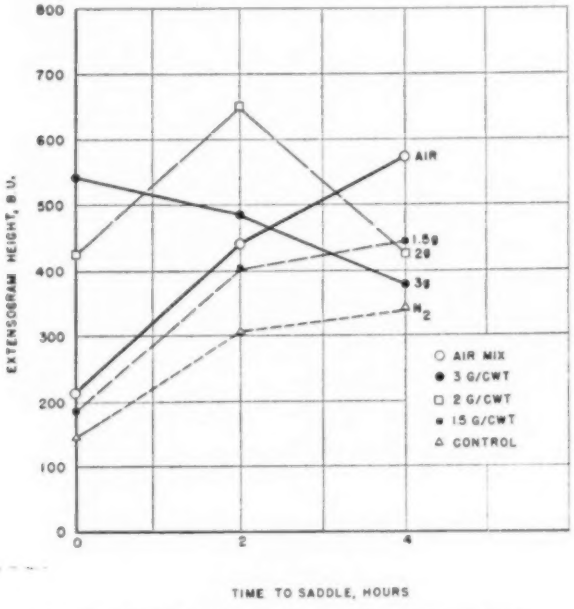

FIG. 13. Effect of chlorine dioxide upon the extensogram height of leavened doughs mixed in nitrogen.

The effect of increments of potassium bromate on unleavened first clear flour doughs is shown in Fig. 10. Here the doughs were mixed in nitrogen and tested after one hour in the saddle.

The addition of 10, 20 and 40 p.p m. of bromate had little effect at zero time. The oxidizing action is reflected in the increases in Extensograph heights with increasing time between mixing and saddling. A curve obtained from an air-mixed untreated dough shows the effect of air as contrasted with bromate in nitrogen-mixed doughs.

Figure 11 shows a similar study with chlorine dioxide. The much more rapid action of this maturing agent is readily seen.

The effects of potassium bromate and chlorine dioxide upon the Extensograph height of leavened doughs (2.5% yeast, 2% salt, 5% sugar, 3% dried skim milk) is shown in Figs. 12 and 13. These doughs were treated in the same way as those for Figs. 10 and 11. The untreated (Control) doughs increased in curve height with an increase in time between mixing and saddling.

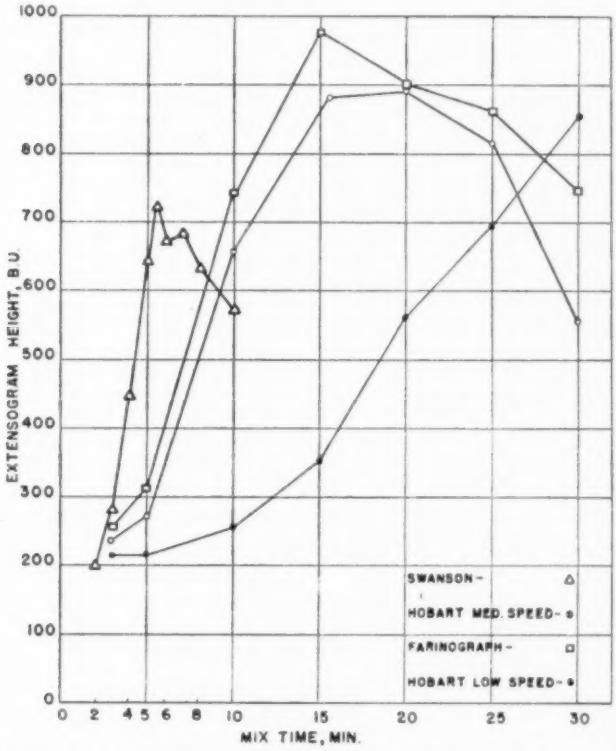

FIG. 14. Effect of mixing variations upon the extensogram height of air mixed doughs.

Again the bromate was less effective with the "no-time" doughs than the chlorine dioxide. With chlorine dioxide, dough breakdown occurred at the higher levels of treatment and the curve heights decreased with an increase in the fermentation time (time to saddle) at the 2 g./cwt. level after two hours and at the 3 g./cwt. level from the start.

Effect of Dough Mixing Time and Atmosphere. The results of Fisher, Aitken and Anderson (2) show that the type of mixer used af-

fected the Extensograph properties of a dough, particularly at the longer reaction times (135 minutes). Unleavened first clear flour doughs containing 2% salt were mixed in the Swanson mixer, the Hobart mixer with a McDuffee bowl, and the Farinograph for the times indicated in Fig. 14. Duplicate 150 g. portions of dough were held at 30°C. for three hours with regular punching, then molded and placed in the saddle. After a saddle time of one hour, Extensograph

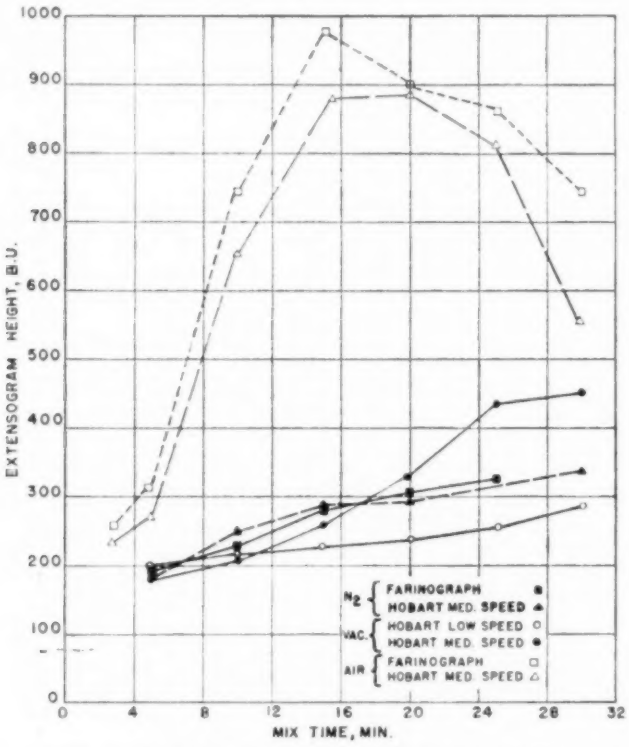

FIG. 15. Effect of mixing variations upon the extensogram height of air, vacuum, and nitrogen mixed doughs.

heights were measured. The results in Fig. 14 show that the most rapid dough development, as measured by Extensograph curve height, was given with the Swanson mixer. This confirms the findings of Fisher *et al.* Development rates in the Farinograph and Hobart McDuffee (medium speed) mixer are about equal. The Hobart mixer at low speed gives slower development.

Doughs were also mixed for varying lengths of time with the Hobart McDuffee mixer at a medium speed in a vacuum of 25 to 26 in.

Hg and under 15 lbs. nitrogen pressure as well as in the Farinograph in nitrogen at atmospheric pressure. The extensograms for the doughs prepared in this way are shown in Fig. 15. At short mixing times there was little difference between the curves for doughs prepared in Hobart mixer or Farinograph in air or in nitrogen or vacuum. As mixing times increased, the effect of the incorporation of oxygen during mixing resulted in a great difference in the dough properties. The

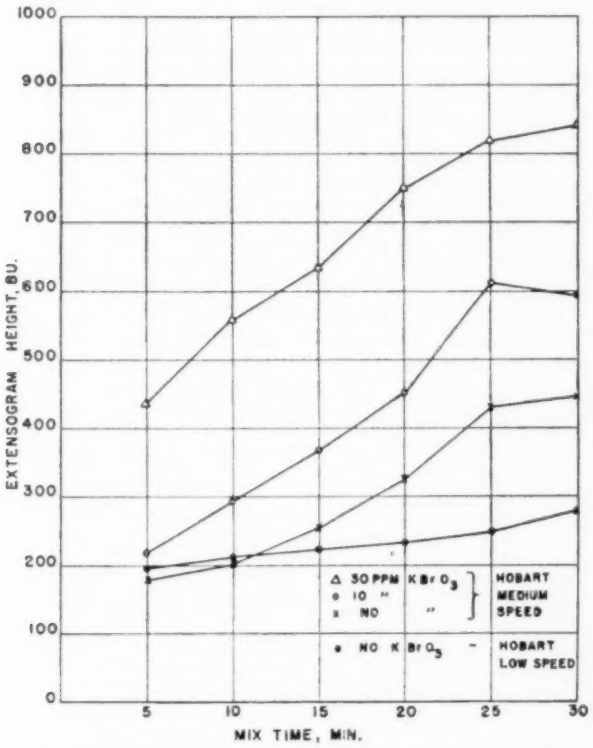

FIG. 16. Effect of mixing in a vacuum upon the action of bromate on dough extensograms.

Extensograph heights increased with mixing time in nitrogen or vacuum. Whether this increase is due to small amounts of oxygen that were not removed from the flour in the inert atmosphere or whether the effect is due only to the mixing action has not been definitely determined.

A test was made with nitrogen, 99.9% pure, and preparing the doughs in a Hobart mixer at medium speed for 25 minutes. An extensogram height of 340 units was obtained, indicating that the purity

of the nitrogen is not a factor. Work in this laboratory has shown that prolonged mixing of doughs in the Farinograph increases their copper content. As copper will act as a flour improver, this may be in part responsible for the change of dough properties with increased Farinograph mixing but does not explain the increases given in the Hobart mixer. The results show that most of the increase in Extensograph height which is caused by increased mixing is due to the incorporation of air.

Effect of Mixing in the Presence of Bromate. A first clear flour was mixed in a vacuum of 26 in. Hg in a jacketed Hobart McDuffee bowl at low speed without the addition of bromate and at medium speed with the addition of 0, 10, and 30 p.p.m. of bromate. Doughs were held at 30°C. using the punching schedule for a three-hour fermentation. They were then molded, placed in the saddle and Extensograph curves drawn after a rest-time of one hour. The effect of mixing time upon Extensograph heights is shown in Fig. 16. Heights of non-bromated doughs increased with increases in mixing time at both low and medium speeds, but the increase was greater at the higher speed. These results and those of Figs. 14 and 15 indicate that the change in dough properties with mixing is related both to the incorporation of air and mechanical action. The former appears to be the more important factor.

The bromated doughs showed greater curve heights than the others and the heights increased with mixing, but the rate of increase was about equal to that for the non-bromated dough at the same mixer speed. This does not show an intensification of bromate action with increased mixing.

Discussion

The changes in the physical properties of flour doughs which are caused by oxidizing agents are related to the type of improver and the amount employed, the time in which it acts, and the physical manipulation of the dough.

Doughs that are not overmixed and are at the proper absorption level show appreciable extensogram heights if tested immediately after mixing.[2] Unoxidized doughs (mixed in nitrogen) rapidly slacken upon standing. Oxidized doughs also rapidly slacken with rest after mixing unless large quantities of a rapidly acting oxidizing agent have been added. Even so, some slackening occurs.

If unoxidized doughs are reworked after a rest period and then tested at an interval after reworking, very low Extensograph curves are obtained. Such treatment of oxidized doughs gives high curve heights depending upon the time interval between the initial mix and the time the dough is molded.

[2] Overmixed doughs may show quite low Extensograph heights if tested directly from the mixer. If mixed in air or oxygen, these doughs often recover with time and reworking to give appreciable Extensograph heights. If mixed in nitrogen, low curve heights will be obtained at any later time. If the dough is greatly overmixed in air or oxygen, the recovery with time and reworking is lessened.

Apparently, little if any oxygen is absorbed by nitrogen-mixed unleavened dough by exposure to air after mixing and during the punching procedure. Leavened doughs mixed in nitrogen show some increase in curve height with fermentation time. The reason for this has not been determined.

Variations in the rate of dough development with different mixers as observed by Fisher, Aitken, and Anderson (2) have been confirmed and appear to be largely due to differences in the amount of air incorporated during mixing. Mixing in inert atmospheres, however, also shows some dough development and this development is related to the mixing speed—indicating that mechanical action is important.

The rate of development of doughs mixed in vacuum in the presence of 10 or 30 p.p.m. of bromate was no greater than without bromate additions. This does not agree with the observation of Baker and Mize (1) that mixing in vacuum increases the effectiveness of bromate.

The effect of dough manipulation coupled with time from mixing cannot be adequately explained by the proteolytic theory of the action of oxidizing agents upon doughs. Dough extensibilities decrease with oxidation. If protease inhibition alone were effective, the extensibility should remain about the same as that of the unoxidized dough. The need for reorientation of the dough before the effect of bromate or oxygen is realized also does not seem reasonable if protease inhibition is responsible for the change in dough properties. The effect of protease activity should be gradual and not occur so dramatically after molding the dough. It also does not appear logical that high levels of oxidizing agents would give a short weak dough if protease inhibition were the mode of action. Both oxidized or unoxidized doughs are only slightly extensible when tested immediately after mixing, but this property is not retained unless the dough is in an oxidized condition. This could be interpreted as indicating proteolytic action in the unoxidized dough were it not for the fact that the extensible dough will again lose its extensibility if reworked again and tested immediately.

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THE PROTEIN COMPOSITION OF FEEBAR, ODESSA, AND PLAINS BARLEY GROWN IN SOUTH DAKOTA [1]

A. W. HALVERSON,[2] J. E. GRAFIUS,[3] and A. L. MOXON [4]

ABSTRACT

Protein composition studies with Feebar, Odessa, and Plains barley of 1.8 to 3.1% of total nitrogen are reported. The data show the effect of three grinding methods (Wiley mill, 1 mm. sieve; hammer mill, 0.5 mm. sieve; and ball mill, 48 hours operation) upon the distribution of nitrogen between the salt-soluble, alcohol-soluble and insoluble protein fractions of barley samples. Fractionation studies of the individual nitrogen components of the salt-soluble fraction are also reported.

Analysis of samples ground with the Wiley or the hammer mill showed that the proportion of the total nitrogen contributed by each protein fraction varied in a regular pattern that depended upon the total nitrogen content of the barley samples. Differences in the rates of increase of the different fractions accounted for the changes in fraction percentages which occurred with change in nitrogen content of the samples. Prominent changes in the proportions of the salt-soluble and the alcohol-soluble fractions occurred with change in the nitrogen content of barley from 1.8 to 2.4% of nitrogen. The insoluble protein fraction represented a more constant percentage of the total nitrogen than did the other fractions; however, this fraction did show a slight decrease in percentage with increase in the nitrogen content of some samples.

In samples of high nitrogen content (2.4–3.1% Nitrogen), the proportions of the protein fractions were little affected by variation in the nitrogen content. It was notable that the kernel nitrogen contents of the high nitrogen samples (Feebar and Odessa barley) also remained practically constant for each variety despite variations in nitrogen percentage. Thus, the data established a varietal relation between kernel nitrogen and protein composition. Because high nitrogen percentages in barley can result from either reduction in kernel size (weight) or from increase in kernel nitrogen, the advantage of relating protein composition to kernel nitrogen as well as to total nitrogen percentage is apparent.

Hammer mill ground samples proved most suitable for protein fractionation studies because efficient extraction of salt-soluble nitrogen was coupled with uniform protein distribution patterns.

The protein composition data for the three varieties (six-row type) were markedly similar at all nitrogen levels studied.

The importance of barley in the brewing industry and as economical livestock feed has prompted several investigators to study the protein

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composition of the grain. As early as 1895 Osborne (6) fractionated and estimated barley proteins by solubility methods. The work indicated that the solubility distribution for barley proteins was similar to that of other cereal grains since the usual four protein fractions (albumin, globulin, prolamine, and glutelin) were present. In 1928 Bishop (2) extensively studied the protein composition of barley as well as the changes which the protein constituents undergo during malting. Bishop's work on protein composition showed that the individual protein components of barley vary in a regular pattern which depends directly upon the total nitrogen content of the grain. Thus, rather accurate calculations of protein composition can be made if the total nitrogen content of the barley sample is known. More recent investigators of the protein composition of barley have included Anderson and Ayre (1), Fink and Kunisch (5), and Urion *et al.* (7). These workers have adequately confirmed the experimental findings initially established by Osborne and Bishop.

Examination of the available literature on barley protein composition indicated the need for more extensive knowledge about the protein composition of barley throughout the barley producing belt in the United States. The new adapted varieties which are constantly being developed also make each geographical area most dependent upon local investigation as to the protein composition of its barley products.

Three six-row barley varieties, Feebar, Plains, and Odessa, were studied. Two of the varieties, Feebar and Plains, were developed from crosses made at the South Dakota Experiment Station in 1936–1937 by S. P. Swenson.[5] Both varieties are well adapted to the South Dakota environment and combine good yield, resistance to stem rust, and stiff straw characteristics. An additional quality of the Plains variety is early maturity. Protein analysis of the two varieties has indicated a medium protein content for the Plains variety and a high content for Feebar. The low diastatic enzyme content of Plains barley limits its malting uses. Nevertheless, this variety holds promise of widespread use in central and western areas of South Dakota where drouth conditions frequently restrict effective crops to the early maturing varieties. Feebar with its high yield and high protein qualities has been recommended as a suitable feed barley for South Dakota. The well established reputation of Odessa barley in this area is largely due to the superior malting quality and satisfactory yields obtained with this variety.

Materials and Methods

Protein fractionation studies were carried out with Feebar, Odessa, and Plains barley samples of different nitrogen content. The samples

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(1950 crop) were grown at various experimental farms in South Dakota by the Agronomy Department of South Dakota State College. The experimental farms are designated as the Redfield Irrigation Farm, Brookings Station Farm, and the Eureka, Highmore, and Cottonwood Substation Farms. The three varieties were grown on adjacent plots on each of the farms.

To study the effect of fineness of grinding upon protein extraction, each barley sample was ground by three different methods. Thus, each sample was divided into three portions which were ground by either a Wiley mill (1 mm. sieve), a hammer mill (0.5 mm. sieve) or a ball mill (48 hours continuous grinding).[6] Nitrogen and moisture determinations were carried out on the samples after completion of grinding.

In the early portion of the study the work was confined to fractionating samples ground with the Wiley mill. The analytical methods were essentially the same as those employed by Bishop (2). The fractionation procedure included extraction of the ground barley with water to remove albumin, proteoses + peptones, and nonprotein nitrogen. Extraction of samples with salt solution (5% potassium sulfate) yielded globulin in addition to the constituents extracted with water. Subtracting the nitrogen content of the water extract from that of the salt extract enabled calculation of globulin nitrogen. Finally, the salt-extracted barley samples were extracted with 70% aqueous ethanol solution to remove alcohol-soluble protein (hordein). The insoluble residual protein (glutelin) of the extracted samples was calculated by difference. Albumin was determined on the water extract by adding 20 ml. of acetate buffer[7] and 60 g. of magnesium sulfate to the extract (200 ml.) and then heating the solution at 82°C. for 40 minutes in a water bath. The precipitated albumin was collected by filtration and nitrogen determined by Kjeldahl analysis. Nonprotein nitrogen was analyzed by the modified method of Blish (4). The proteose + peptone fraction was calculated by subtracting the sum of the nitrogen of the albumin and the nonprotein nitrogen fractions from the total nitrogen of the water extract.

When study of the ball- and hammer-milled samples was initiated, the fractionation procedure was shortened by eliminating the water extraction step. In order to compensate for the diminished data which resulted from the change, a trichloroacetic acid precipitation procedure was added to the determinations performed on the salt extract (3). By determining the quantity of albumin + globulin nitrogen precipitated by 2.27% trichloroacetic acid, it was again

[6] Ball-milled samples were ground with the Wiley mill (1 mm. sieve) before ball-mill grinding.
[7] The acetate buffer was composed of equal volumes of normal solutions of sodium acetate and acetic acid. The pH of the buffer was 4.6.

possible to calculate the nitrogen represented by the proteose + peptone fraction. The calculation merely employed subtraction of the combined nitrogen of the albumin + globulin and the nonprotein nitrogen fractions from the total nitrogen of the salt extract.

Extracts from some samples which were ground with the ball mill did not show satisfactory precipitation with trichloroacetic acid since the usual clarification of the filtrates was not evident. Thus, it was necessary to discard the albumin + globulin and the proteose + peptone data for the ball-milled samples. In every instance satisfactory trichloroacetic acid precipitation was obtained with hammer-milled samples. Since no difficulty was encountered with the nonprotein nitrogen determinations on the ball-milled samples, those data were included in the report. Nonprotein nitrogen analyses of both Wiley- and ball-milled samples gave similar values for comparable samples. Thus, the need for further study of the effect of grinding methods upon this constituent was obviated and no nonprotein nitrogen determinations were carried out on hammer-milled samples. With hammer-milled samples, the differential calculation of the proteose + peptone fraction was carried out by employing the average nonprotein nitrogen values obtained from comparable Wiley- and ball-milled samples.

The centrifugation procedure employed in the fractionation studies was carefully controlled. In the case of the Wiley-milled samples, the centrifugation force was 1,370 $\times$ *g* (2,550 r.p.m.) and for the hammer- and ball-milled samples the force was 1,570 $\times$ *g* (2,730 r.p.m.). The customary procedure for removing the water, salt, and alcohol extracts from the centrifuged samples was merely to decant the supernatant extract. However, when separation of aqueous extracts from insoluble particles by decantation proved difficult, a siphoning procedure was carried out. Even with the siphoning, it was often difficult to make a clear cut separation between insoluble and soluble barley constituents with some ball-milled samples. With these samples centrifugation caused the insoluble barley residue to settle as a loose stratified mass rather than as a compact one suitable for a decanting operation. Samples which had a fine powdery texture did not centrifuge properly, while those with a coarse granular consistency could be centrifuged without difficulty during the extraction procedures. Obviously the particle size of some of the ball-milled samples was too fine to enable proper centrifugation with the equipment employed.

The trunnion-type centrifuge head was employed throughout the study. Although conical-type centrifuge heads would have enabled greater centrifugation forces than were possible with the trunnion-type, the tube breakage encountered with the conical-type at high

speed was undesirable. However, it is quite possible that greater centrifugation forces would have promoted more effective separations with ball-milled samples.

All fractionation analyses were performed in duplicate. Suitable blank Kjeldahl nitrogen determinations were carried out to eliminate possible contamination from reagents employed in the protein fractionations. Thousand-kernel weights were determined by counting and weighing 100 kernel samples in triplicate. A high degree of reproducibility was observed in all determinations.

Results

Protein fractionation data for Feebar, Odessa, and Plains barley are shown in Table I. The table shows that the amount of each protein fraction (salt-soluble, alcohol-soluble, and insoluble) in barley increases as the total nitrogen content of the barley increases. Further, the table shows that grinding by hammer or ball mill, as contrasted with the Wiley mill, caused a marked increase in the salt-soluble fraction and a comparable decrease in the insoluble protein fraction (glutelin). The alcohol-soluble protein fraction (hordein) was little affected by the method of grinding employed for the samples.

When the protein fractions for each variety are plotted as percentages of the total nitrogen (Fig. 1), the data show a regular pattern which relates the fraction percentages to the total nitrogen content of the barley. The similarity of protein composition between the three varieties is evident from the Wiley and hammer mill data presented in the figure. Examination of the Wiley and hammer mill curves further shows that the hordein and salt-soluble nitrogen fraction percentages in medium nitrogen samples were much affected by changes in the total nitrogen content of the samples. However, with high nitrogen samples (2.4 to 3.1% Nitrogen or 15.0 to 19.4% protein), no notable changes in fraction percentages occurred with variation in the total nitrogen content.

The data obtained for samples ground with the ball mill showed a less uniform pattern of distribution of protein fractions than that obtained with the other grinding methods (Fig. 1). The failure of the ball-milled samples to yield data which showed a uniform relationship between the total nitrogen and the percent distribution of each of the fractions was apparently due to variability in fineness of grinding.

Table II shows nitrogen content and protein composition data on single barley kernels. The kernel nitrogen for each variety was significantly higher in the Brookings, Highmore, and Cottonwood samples than in the Redfield and Eureka samples. This increase in kernel nitrogen between different samples of the same variety was largely due

TABLE I

NITROGEN FRACTIONS IN BARLEY OF DIFFERENT TOTAL NITROGEN CONTENTS WHEN GROUND BY DIFFERENT METHODS, AS PERCENTAGES OF DRY MATTER

Name of Variety Type of Grinding Mill Location where Grown	Total Nitrogen	Barley Protein Fractions		
		Salt-Sol. Nitrogen	Alcohol-Sol. Nitrogen	Insol. Nitrogen
	%	%	%	%
Feebar				
Wiley mill				
Redfield	1.97	0.54	0.56	0.87
Eureka	2.01	0.53	0.54	0.94
Brookings	2.58	0.60	0.93	1.05
Highmore	2.79	0.65	0.99	1.15
Cottonwood	3.10	0.70	1.09	1.31
Hammer mill				
Redfield	1.97	0.67	0.59	0.71
Eureka	2.01	0.69	0.60	0.71
Brookings	2.58	0.78	0.95	0.85
Highmore	2.79	0.82	1.06	0.91
Cottonwood	3.10	0.91	1.22	0.97
Ball mill				
Redfield	1.97	0.66	0.55	0.76
Eureka	2.01	0.73	0.59	0.69
Brookings	2.58	0.82	0.96	0.80
Highmore	2.79	0.82	1.11	0.87
Cottonwood	3.10	0.99	1.19	0.92
Odessa				
Wiley mill				
Redfield	1.79	0.49	0.51	0.80
Eureka	1.97	0.55	0.55	0.87
Brookings	2.38	0.55	0.82	1.01
Highmore	2.44	0.57	0.82	1.06
Cottonwood	2.79	0.64	1.02	1.13
Hammer mill				
Redfield	1.79	0.71	0.52	0.56
Eureka	1.97	0.73	0.58	0.66
Brookings	2.38	0.73	0.89	0.76
Highmore	2.44	0.77	0.88	0.79
Cottonwood	2.79	0.85	1.05	0.89
Ball mill				
Redfield	1.79	0.64	0.50	0.64
Eureka	1.97	0.75	0.60	0.62
Brookings	2.38	0.87	0.79	0.72
Highmore	2.44	0.81	0.82	0.82
Cottonwood	2.79	0.77	1.02	1.00
Plains				
Wiley mill				
Redfield	2.22	0.54	0.68	1.01
Eureka	2.38	0.56	0.74	1.08
Brookings	2.53	0.56	0.88	1.09
Highmore	2.81	0.64	1.06	1.11
Cottonwood	2.85	0.63	1.00	1.23

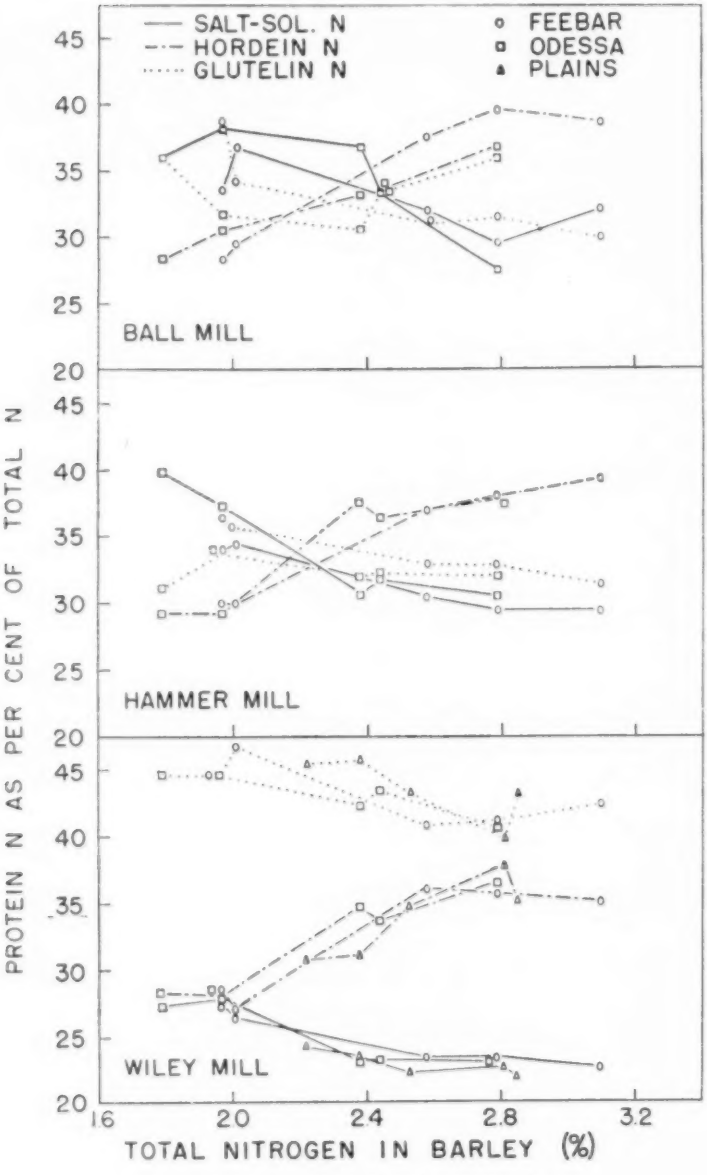

FIG. 1. Effect of the method of grinding and the nitrogen content of barley upon protein composition.

to greater amounts of alcohol-soluble protein per kernel. A further observation was that the kernel nitrogen content of samples of Feebar and Odessa barley grown at the Brookings, Highmore, and Cottonwood stations showed little variation within varieties even though the nitrogen content (% nitrogen) in the samples varied considerably. This increase in sample nitrogen percentage without increase in kernel

TABLE II

KERNEL WEIGHTS AND CALCULATED PROTEIN COMPOSITION [1] OF SINGLE KERNELS WITH BARLEY SAMPLES OF DIFFERENT NITROGEN CONTENT

Location where Grown	Nitrogen in Samples[2] (%)	Weight per 1,000 Kernels[3] (g.)	Protein Composition of Kernel			
			Salt-Sol. N (mg.)	Alcohol-Sol. N (mg.)	Insol. N (mg.)	Total N (mg.)
Feebar						
Redfield	1.97	42.2	0.26	0.23	0.28	0.77
Eureka	2.01	38.1	0.24	0.21	0.25	0.70
Brookings	2.58	40.9	0.29	0.36	0.32	0.97
Highmore	2.79	36.2	0.27	0.35	0.30	0.92
Cottonwood	3.10	32.4	0.27	0.36	0.29	0.93
Odessa						
Redfield	1.79	35.9	0.24	0.17	0.18	0.59
Eureka	1.97	34.7	0.23	0.18	0.21	0.62
Brookings	2.38	34.4	0.23	0.28	0.24	0.74
Highmore	2.44	31.1	0.22	0.25	0.22	0.69
Cottonwood	2.79	29.9	0.23	0.29	0.24	0.76
Plains						
Redfield	2.22	36.9	—	—	—	0.76
Eureka	2.38	35.5	—	—	—	0.77
Brookings	2.53	38.6	—	—	—	0.88
Highmore	2.81	38.7	—	—	—	0.98
Cottonwood	2.85	32.6	—	—	—	0.85

[1] Hammer mill fractionation data were employed for the protein composition calculations.
[2] Moisture-free basis.
[3] Air-dry basis.

nitrogen resulted from reduction in kernel weights of the high nitrogen samples of the two varieties.

Table III shows data on the water-soluble and salt-soluble nitrogen components of the barley samples. Although the fractionation procedure was modified (shortened) after completion of the Wiley mill samples from the Redfield, Brookings, Highmore, and Cottonwood stations, the data presented are still of much importance. Examina-

TABLE III

SALT-SOLUBLE NITROGEN FRACTIONS IN BARLEY GROWN AT DIFFERENT STATIONS, AS PERCENTAGES OF THE TOTAL NITROGEN

Type of Grinding Salt-soluble N. Fraction	Redfield (%)	Eureka (%)	Brookings (%)	Highmore (%)	Cotton-wood (%)
Feebar Barley					
Total Nitrogen Content	1.97	2.01	2.58	2.79	3.10
Wiley mill					
Total salt-soluble N	27.4	26.3	23.4	23.4	22.6
Albumin N	6.6	—	4.1	3.9	3.4
Albumin + globulin N	15.0	15.4[1]	13.0	12.0	11.9
Proteose + peptone N	7.3	6.8[1]	5.9	5.4	5.0
Nonprotein N	5.0	4.1	4.5	6.1	5.7
Hammer mill					
Total salt-soluble N	33.8	34.6	30.3	29.4	29.4
Albumin + globulin N	21.5[1]	22.3[1]	19.1[1]	17.6[1]	17.2[1]
Proteose + peptone N	7.3[1]	7.6[1]	6.5[1]	6.1[1]	6.3[1]
Ball mill					
Total salt-soluble N	33.5	36.5	31.8	29.3	31.8
Nonprotein N	5.1	5.1	5.0	5.4	6.2
Odessa Barley					
Total Nitrogen Content	1.79	1.97	2.38	2.44	2.79
Wiley mill					
Total salt-soluble N	27.2	27.8	23.1	23.2	23.0
Albumin N	7.0	—	4.2	4.3	3.7
Albumin + globulin N	13.3	15.9[1]	13.6	12.8	14.1
Proteose + peptone N	7.7	6.8[1]	5.4	5.3	4.3
Nonprotein N	6.3	5.1	4.1	5.1	4.6
Hammer mill					
Total salt-soluble N	39.8	37.1	30.6	31.7	30.4
Albumin + globulin N	25.5[1]	23.5[1]	19.0[1]	19.2[1]	18.4[1]
Proteose + peptone N	8.5[1]	8.3[1]	7.2[1]	7.4[1]	7.3[1]
Ball mill					
Total salt-soluble N	35.9	38.0	36.6	33.1	27.7
Nonprotein N	5.2	5.6	4.6	4.8	4.8
Plains Barley					
Total Nitrogen Content	2.22	2.38	2.53	2.81	2.85
Wiley mill					
Total salt-soluble N	24.1	23.4	22.2	22.7	21.9
Albumin N	5.1	—	4.5	4.1	3.5
Albumin + globulin N	13.7	13.9[1]	13.5	13.1	13.3
Proteose + peptone N	6.1	5.8[1]	5.1	5.4	4.8
Nonprotein N	4.3	3.7	3.6	4.2	3.8

[1] Samples that were analyzed for albumin + globulin and proteose + peptone nitrogen by the trichloroacetic acid precipitation method.

tion of the Wiley mill sample data shows that the proportion of the total nitrogen contributed by the albumin as well as by the proteose + peptone fraction decreased as the nitrogen content of the samples increased. Globulin nitrogen either remained a constant percentage of the total nitrogen or else tended to increase slightly in the high nitrogen samples. Determination of the albumin + globulin and the proteose + peptone components in hammer-milled samples by the modified procedure also showed that the percentages of these components tended to decrease with increase in the total nitrogen content of the samples. Nonprotein nitrogen values in both Wiley- and ball-milled samples remained a constant percentage of the total nitrogen except for minor fluctuations.

The increased nitrogen obtained in the salt-soluble extract from more finely ground samples (hammer versus Wiley mill) largely represented the albumin + globulin component. The data do not show the extent of the increase in extracted nitrogen contributed by the separate albumin and globulin constituents. Also a greater amount of proteose + peptone nitrogen was present in the extract from the more finely ground samples. The nonprotein nitrogen contents of extracts from coarse (Wiley mill) and fine (ball mill) ground samples were very similar.

No notable differences in the percentages of the different components of the salt-soluble fraction were evident between the three varieties studied. It is recognized that more precise analytical methods and a greater number of samples are essential for ascertaining whether slight differences in protein composition may be related to varietal factors rather than to numerous other variables which are evident in the estimation of biological constituents.

Discussion

The advantage of grinding samples with the hammer mill rather than with the Wiley mill (1 mm. sieve) or the ball mill is apparent. The data in Table I demonstrated the incomplete extraction of the salt-soluble nitrogen that resulted with samples ground with the Wiley mill. The fact that the salt-soluble nitrogen extracts from hammer- and ball-milled samples approached a common value indicated that both grinding methods produced samples which enabled efficient extraction of salt-soluble nitrogen. The superiority of the hammer-mill over the ball-mill method of grinding is shown by the data presented in Fig. 1. The distribution of the protein constituents of hammer-milled samples showed a regular pattern which correlated total nitrogen with percentages of the separate protein fractions. In contrast, the ball-milled samples yielded extracts of such variable

nitrogen content that accurate correlation between total nitrogen and percentages of separate constituents was precluded.

A uniform degree of grinding between different samples is obviously essential for attaining comparable results in protein fractionation. Samples ground with the hammer mill should have been comparable since the sieve (0.5 mm.) would standardize maximum particle size. In the case of the ball-milled samples, no assurance of standardized particle size between different samples was insured after prolonged grinding effected by crushing with rolling stones. The physical appearance of the different ball-milled samples combined with fractionation observations indicated that grinding with the ball mill produces samples lacking the desired uniformity of particle size.

The data on barley of 1.8 to 2.4% of nitrogen adequately confirmed the studies of Bishop (2) and Anderson and Ayre (1), since increase in the nitrogen content of the samples was accompanied by an increase in the proportion of the total nitrogen contributed by hordein and a decrease in the proportion contributed by salt-soluble nitrogen (Fig. 1). The quantitative distribution values were more in agreement with the data obtained by the Canadian investigators (Anderson and Ayre) than with that obtained by the English (Bishop).

With barley samples of higher nitrogen content (2.4–3.1% nitrogen), changes in the proportion of the hordein and salt-soluble nitrogen were no longer clearly evident with increase in nitrogen content of the samples. It was notable that the kernel nitrogen of the high nitrogen Feebar and Odessa samples was also essentially constant (Table II). Thus, the present report establishes that high nitrogen samples which vary in nitrogen percentage but not in actual kernel nitrogen contain almost constant proportions of the different protein fractions. Until studies are conducted on high nitrogen samples in which increases in nitrogen percentage actually reflect increases in kernel nitrogen content, no overall conclusion in regard to the protein composition of high protein barley can be made. Future investigators who study the composition and nutritive value of the proteins from high and low nitrogen barley samples should compare difference in terms of kernel nitrogen as well as nitrogen percentage since kernel nitrogen may well be a more exact standard for predicting protein composition than is nitrogen percentage.

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DETERMINATION OF SULFHYDRYL GROUPS IN FLOUR BY MEANS OF *o*-IODOSOBENZOATE AND RADIOACTIVE IODINE [1, 2]

P. NORDIN [3] and E. Y. SPENCER [4]

ABSTRACT

The original method for the determination of sulfhydryl groups in certain proteins by means of *o*-iodosobenzoate had to be modified when used for flour due to the interference of starch. This modification consisted in determining the excess iodosobenzoate by the addition of sodium iodide containing radioactive iodide. The free iodine liberated was adsorbed by the starch and the unreacted sodium iodide was then determined in the supernatant by a dip counter. By this method results were obtained which indicated that the sulfhydryl content ranged from 1.2×10^{-6} equivalents per gram for a patent flour, 1.6×10^{-6} for a break flour to 2.3×10^{-6} for the red dog portion. Accelerated ageing experiments indicated no significant decrease in the sulfhydryl content. However, in each ageing experiment less *o*-iodosobenzoate (used as an improving agent) was required for optimum loaf volume as ageing progressed.

The improving effect of certain oxidizing agents and the deleterious effect of some reducing agents on the baking quality of flour is well known. The improving effect has been ascribed to the oxidation of sulfhydryl groups to disulfides (9). The small amount of improver required indicates that if oxidation of sulfhydryl groups is the mechanism very low concentrations of sulfhydryl groups are involved. The determination of such concentrations presents difficulties, especially since the methods generally used are known to be nonspecific (1, 8). *o*-Iodosobenzoate, however, has been found to be specific in the presence of a considerable number of amino acids (4). In addition,

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[2] Contribution from the Department of Chemistry, University of Saskatchewan, Saskatoon, Saskatchewan, with financial assistance from the National Research Council and the Saskatchewan Agricultural Research Foundation. Published as paper No. 288 of the Associate Committee on Grain Research, of the National Research Council. Presented in part to the Chemical Institute of Canada Conference, June 20, 1950.
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o-iodosobenzoate functions as an improver (7, 10). In some experiments carried out here, no response could be obtained from the reduced form, *o*-iodobenzoate, while concentrations of *o*-iodosobenzoate caused drastic changes in loaf volumes. These findings support the generally accepted view that sulfhydryl groups play an important part in the improver mechanism.

In determining how much *o*-iodosobenzoate reacts with a flour slurry, the difficulty of the low concentration is aggravated by the phenomenon of adsorption. Normally the excess *o*-iodosobenzoate is determined by reaction with sodium iodide and the liberated iodine is titrated with standard thiosulfate or standard arsenite. In this case the high concentration of starch adsorbs some or all of the iodine. The difficulty cannot be overcome by centrifuging the slurry and analyzing the supernatant for iodosobenzoate, for in this case some of the *o*-iodosobenzoate is adsorbed on the centrifuged material. In an attempt to overcome these difficulties it was decided to adopt a radioisotope technique.

In studying ageing effects, a method capable of determining the sulfhydryl content of flour slurries was desired and it was felt the *o*-iodosobenzoate might be of value. The essential difference in the method reported here is in the determination of the excess *o*-iodosobenzoate over that required for reaction with the flour. Following synthesis of the reagent, a method was developed to determine the apparent-SH content of flour slurries, overcoming adsorption interference in estimating the excess iodosobenzoate by use of radioactive iodine. The method was applied to different grades of flour, stored for varying periods at different temperatures. The effect of iodosobenzoate was compared with potassium bromate in baking response studies.

Preparation of *o*-Iodosobenzoate

Wilgerodt (11) first prepared *o*-iodosobenzoic acid from *o*-iodobenzoic acid by chlorination and Lucas and Kennedy (6) prepared *o*-iodosobenzene by this method. Greenbaum (3) described the preparation of *o*-iodobenzoic acid from anthranilic acid by diazotization followed by a Sandmeyer reaction with potassium iodide. Adopting the procedure of Greenbaum and Lucas and Kennedy, a product was easily obtained in 60% overall yield (99.7% purity by an iodometric titration). The chlorination steps offer some advantages over the direct potassium permanganate oxidation of *o*-iodobenzoic acid. The product is more easily isolated and in somewhat better yields.

Determination of *o*-Iodosobenzoate

Materials. (1) Geiger solution counter. Herbach and Rademan Co., with a C.S.R.D.E. Scaling outfit.

(2) Centrifuge (International Size 1) 50 ml. centrifuge tubes with about two-thirds of the upper part cut off.

(3) High speed stirrer. An air stirrer fitted with a glass rod agitator which is long enough to reach to the bottom of the tube.

(4) Iodide solution. 0.0075 *N* sodium iodide in 0.3 *N* hydrochloric acid and enough active iodine[5] as sodium iodide so that a 2 ml. aliquot has an activity of about 100 clicks per minute. One click is equal to 128 counts per minute. This solution is freshly made up and sodium bisulfite crystals or a drop of bisulfite solution added to reduce the iodine which forms from atmospheric oxidation. The solution is

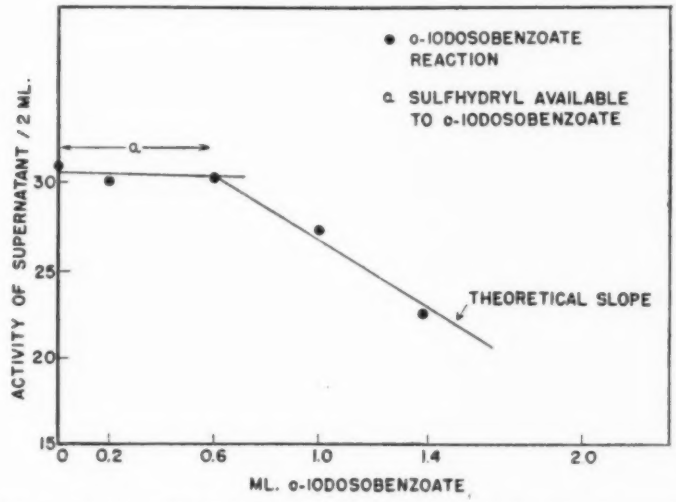

FIG. 1. Determination of sulfhydryl groups in flour slurries available to *o*-iodosobenzoate by measuring excess reagent with radioactive iodine.

then boiled gently for about five minutes to dispel all excess sulfur dioxide. It is then stored in a brown bottle.

(5) Sodium *o*-iodosobenzoate (0.00210 *N*). Adjust to about pH 9 with 0.1 *N* sodium hydroxide solution.

Procedure. Eight 1g. samples of flour were weighed. Three milliliters of distilled water were then added to a centrifuge tube and a flour slurry made by adding one of the weighed flour samples with vigorous stirring. After allowing the slurry to stand exactly two minutes, 1 ml. of iodide solution (containing radioactive iodide) was added. The mixture was then stirred vigorously and after 45 seconds centrifuged at 2,500 r.p.m. for one minute. The activity was then determined in a 2

[5] NaI^{131} obtained from National Research Council, Isotopic Division, Chalk River, Ontario, Canada.

ml. portion of the supernatant. The procedure was repeated with the remaining seven samples but replacing part of the water with different levels of *o*-iodosobenzoate solution. The activity of the 2 ml. supernatant sample in each case was plotted against milliliters of *o*-iodosobenzoate added. A straight line was drawn through those points which had the same activity as the blank and then a straight line was drawn through those points which showed a drop in activity. The intersection of the two lines gave the amount of *o*-iodosobenzoate which reacted with the flour (see "a" Fig. 1). Any *o*-iodosobenzoate adsorbed by the flour would no doubt react quickly with the iodide solution.

Results

Application to a Patent Flour. In Fig. 1 the results obtained from a 1-year-old patent flour are plotted. The *o*-iodosobenzoate is 0.00210 *N*, the sodium iodide is 0.00680 *N*. The intersection of the two lines occurs at 0.60 ml. *o*-iodosobenzoate or for 1.2×10^{-6} equivalents per gram of flour. The line marked "theoretical slope" is calculated as follows. Neglecting adsorption of iodide ions, any drop in activity after 0.60 ml. *o*-iodosobenzoate is due to iodine being formed and adsorbed on the starch and hence removed from the solution on centrifuging. Hence $1.40 - 0.60 = 0.80$ ml. *o*-iodosobenzoate $\equiv \frac{0.80 \times 0.00210}{0.00680} \equiv 0.247$ ml. of iodide solution. One milliliter sodium iodide in the sample with no *o*-iodosobenzoate gave a count of 30.0 clicks per minute in the supernatant. If only iodine is adsorbed by the starch, then 0.8 ml. of *o*-iodosobenzoate should cause a drop in activity of $0.247 \times 30.0 = 7.4$ clicks per minute. The line with this required slope was drawn as shown. Agreement with the theoretical slope and the observed points, is taken to mean that iodine can be quantitatively separated from iodide solution in this manner. This point has been verified with pure wheat starch.

Reaction of Iodine with Flour

An alternate explanation of the quantity "a" in Fig. 1 might be that iodine is being reduced to iodide by reducing groups in the flour, and that the quantity "a" is a measure of these groups available to iodine, but not to *o*-iodosobenzoate. Some workers have determined sulfhydryl in protein by titrating with iodine in acid solution (1). The chief difficulties were that other groups were affected and sulfhydryl groups were oxidized further—possibly to sulfones, although oddly enough disulfides were not affected. Freilich (2) has demonstrated that the quantity of iodine consumed by flour extracts increases with an increase in pH.

To investigate the degree of reaction of iodine with flour slurries as a function of hydrogen ion concentration the following experiment was performed: 1 ml. of 0.00740 *N* *o*-iodosobenzoate and 1 ml. of 0.00890 *N* sodium iodide solution plus 0.01 ml. of 1 *N* hydrochloric acid (pH 3.5) were placed in a centrifuge tube. After the reaction was complete (two minutes), 1 ml. of a citric acid-disodium phosphate buffer of the desired pH was run in, 1 ml. of water added, and 1 g. of flour incorporated with vigorous stirring. After 45 seconds the slurry was centrifuged for one minute and the supernatants poured off into test tubes and counted. Figure 2 shows the variation in count with pH. It was noticed that the residues at the low pH values retained their dark blue color for several days whereas the ones at higher pH turned colorless in a few minutes. The iodine in the starch complex is not held strongly enough to prevent it reacting with protein. Evidently this is oxidation and not substitution, since almost all of the iodine was converted to a water-soluble material—presumably iodide. The theoretical activity value for no conversion of iodine to iodide (from the normality and known activity of the iodide) is calculated and marked on this graph. Even at pH 1.6 a slight amount of iodine was converted to iodide.

Influence of pH on the Reaction of *o*-Iodosobenzoate with Flour

Slurries of 1 *N* hydrochloric acid or 0.1 *N* sodium hydroxide with one gram of flour and 0.5 ml. of 0.0101 *N* *o*-iodosobenzoate were made (this is a definite excess). The amounts of hydrochloric acid or sodium hydroxide were chosen so as to provide a wide range of pH values. The total volume of liquid was adjusted to 4.0 ml. with water. After a reaction time of one minute, enough hydrochloric acid was added to lower the pH to 1.6 and 1 ml. of 0.00892 *N* sodium iodide (containing radioactive iodide) added simultaneously. (This was done to lower the pH below 3 and thus minimize the reduction of iodine to iodide.) The slurry was mixed and centrifuged as before and the supernatants counted. The results are also shown in Fig. 2. The increase in activity of the supernatant with decrease in pH indicates that less iodide was converted to iodine and consequently less excess *o*-iodosobenzoate has been used up, but from pH 4.0 to 7.0 the results were very nearly constant. Hellerman (4) cautions against having a high acidity when *o*-iodosobenzoate is used. This experiment shows that pH 4.0 seems to be the lower limit for gluten.

Use of Active *o*-Iodosobenzoic Acid

To provide evidence for the fact that the quantity "a" in Fig. 1 is due to oxidation by *o*-iodosobenzoic acid and not iodine, active *o*-iodoso-

benzoic acid was used. This was prepared by adding NaI^{131} with the potassium iodide in the Sandmeyer step mentioned under preparation. When active *o*-iodosobenzoate reacts with sulfhydryl groups active *o*-iodobenzoate will be formed in amounts equal to the amount of sulfhydryl groups oxidized. Some of this iodobenzoate will be adsorbed on the flour and be removed on centrifuging. However if the amount of iodosobenzoate remaining in the supernatant is determined by titration and the amount of activity (due to iodoso and iodobenzoate) by counting, the concentration of sulfhydryl groups will be given by the difference in apparent concentration of the iodosobenzoate by

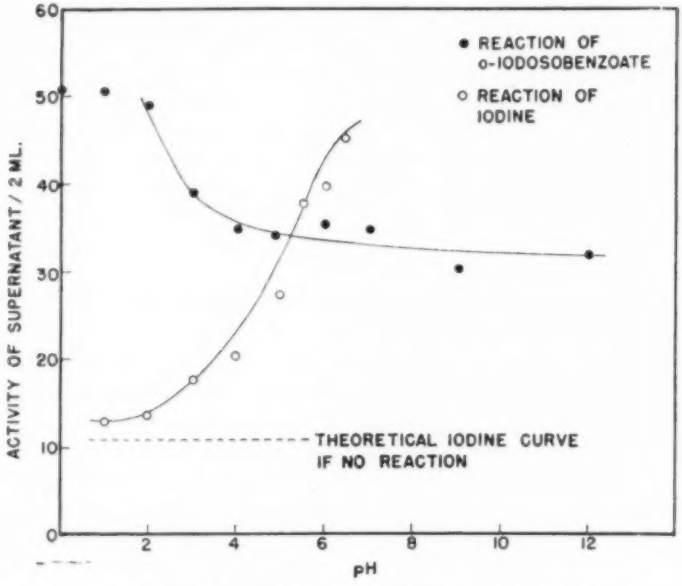

FIG. 2. Influence of pH on reaction of *o*-iodosobenzoate and iodine with flour.

count plus the amount of iodobenzoate adsorbed minus the concentration of the iodosobenzoate given by titration. Many samples were studied over a wide range of concentrations of *o*-iodosobenzoic acid. A 2 ml. microburette which could be read to the nearest 0.01 ml. was used for the titration with potassium iodide and standard sodium thiosulfate. No buffer was used as the natural buffering action of the flour maintains a pH of 5.8 to 6.2. Buffers caused cloudy solutions which interfered with the endpoint of the titration. There is a considerable amount of reducing matter in the water-soluble portion of the flour, consequently before titration, 1 ml. of *o*-iodosobenzoate was

added to the aliquot and after one minute potassium iodide and 1 ml. of 0.5 *M* citric acid. The iodine was then back titrated with 0.0245 *N* sodium thiosulfate. In this way a more comparable blank was obtained for all the samples. The count was made within an error of 4%. Eight-gram samples of a patent flour and 32 ml. of liquid were used so that a 10 ml. aliquot of supernatant could be used for titration.

One milliliter of 0.00564 *N* *o*-iodosobenzoate is equivalent to 0.23 ml. of 0.0245 *N* thiosulfate. When 1 ml. of this *o*-iodosobenzoate is added to a 10 ml. aliquot of the supernatant of a flour slurry, 0.185 ml. thiosulfate were used. Therefore the water soluble reducing matter in this dispersion was: $\frac{(0.23-0.185)\times 0.0245}{1{,}000}\times\frac{32}{10}\times\frac{1}{8}=0.44\times10^{-6}$ eq./g. flour.

With the addition of small amounts of *o*-iodosobenzoate to the slurry the titre approached the theoretical value of 0.23 ml. of thiosulfate. Further additions of *o*-iodosobenzoate to the slurry resulted in only small increases in the titre until 2.5 ml. were added. Two and five-tenths milliliters is 1.75×10^{-6} equivalents, a definite excess over 1.2×10^{-6} equivalents, which is the value "a" found for sulfhydryl groups. Evidently addition of *o*-iodosobenzoate results first in the oxidation of water-soluble material. If the water-soluble material were oxidized by iodine rather than *o*-iodosobenzoate one could expect a gradual increase in the thiosulfate titre rather than a levelling off at the theoretical value of 0.23 ml. Hence, the water-soluble reducing material is probably sulfhydryl as well.

Concentration in solution was calculated assuming that water-soluble reducing matter is sulfhydryl in nature. Calculations were made on the basis of 4.0 ml. so that 1 g. of flour could be considered, i.e., flour to water ratio of 1:4. For example,

Concentration in solution by count—2 ml. of 0.00564 *N* *o*-iodosobenzoate had an activity of 7.48 cl/min. Therefore the concentration per 4 ml. of solution was

$$\frac{1.33}{7.48}\times\frac{0.00564\times 4.0}{1{,}000}=4.0\times10^{-6}\text{ equivalents.}$$

Concentration in solution by titre—0.54 − 0.23 = 0.31 ml. thiosulfate. Therefore the concentration per 4 ml. of solution is

$$\frac{0.31\times 0.0245}{1{,}000}\times\frac{4}{10}=3.04\times10^{-6}\text{ equivalents.}$$

The difference is $(4.0 - 3.04) \times 10^{-6} = 0.96 \times 10^{-6}$ equivalents.

In a separate experiment the adsorption of *o*-iodobenzoic acid was

determined. Reproducibility was poor at such low concentrations, and an average value was taken as 0.2×10^{-6} equivalents. This value was added to the difference between concentration by count and concentration by titre to give "Sulfhydryl/gram flour." The values, although not very reproducible, represented a positive value in fair agreement with the 1.2×10^{-6} equivalents found previously for the sulfhydryl content.

Application of the Method to Various Flours

The general procedure outlined for the determination of sulfhydryl groups was applied to various flours. The value for sulfhydryl was taken as the point where concentration in solution began to fall. The error was the total uncertainty in the position of this point.

TABLE I

SULFHYDRYL CONTENT OF VARIOUS FLOURS

Wheat Sources	Flour Grade	Age[1]	Protein Content	SH Content
			%	*eq./g. flour* ($\times 10^{-6}$)
Thatcher	Straight	1½ months	16.0	1.30
Thatcher	Straight	1½ months	11.9	1.20
Piebald mixture	Straight	1 month	8.7	1.05
Soft wheat mixture		1 month	14.2	1.10
Thatcher[2]	Straight	2 months	—	1.20
Thatcher[2]	Break	2 months	—	1.60
Thatcher[2]	Red dog	2 months	—	2.60
—[3]	Straight	13 years	—	1.05
—[3]	Straight	2 years	19.2	1.15
—[3]	Straight	2 years	11.0	1.10

[1] The time between milling and the experiment.
[2] Flour samples of different purity were milled from the same wheat.
[3] Hard red spring wheat mixture.

The results are given in Table I. The sulfhydryl content was not proportional to the protein content, and was not significantly different between samples of straight flour tested, regardless of age. There was a significant increase in sulfhydryl with decrease in grade of flour.

Baking Experiments

A second break flour showing an optimum response to bromate at 5 mg. of potassium bromate per 100 g. flour was used. The flour was divided into portions and stored under various conditions: 40°C., room temperature, and refrigerated at 0° to 10°C. The response of this flour to *o*-iodosobenzoate was determined by a baking test using a semi-rich formula which contained a hydrogenated vegetable shorten-

ing, nonfat milk solids and ammonium dihydrogen phosphate. The volumes of the baked loaves were measured when various quantities of *o*-iodosobenzoate solution were incorporated into the dough and are shown in Fig. 3.

At the time when this flour was received the method for the determination of sulfhydryl groups in flour had not been developed so that a sulfhydryl determination could not be made paralleling the first baking tests. However, a sulfhydryl determination was made on the flours after storing for $1\frac{1}{2}$ years in the refrigerator and at 40°C. The values

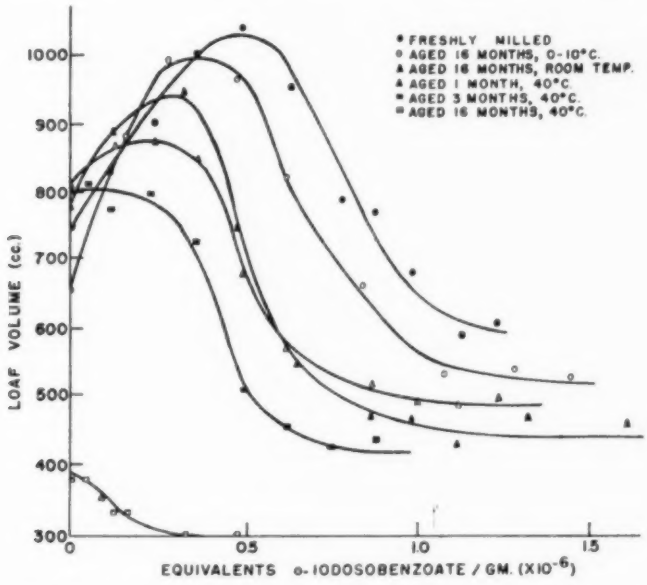

FIG. 3. The effect of *o*-iodosobenzoate on loaf volume of a flour under various conditions of ageing.

obtained were not significantly different from that of the flour which had been stored at room temperature, i. e. $(1.2 \pm 0.15) \times 10^{-6}$ eq./g.

The maximum response to *o*-iodosobenzoate from the freshly milled second break flour occurred at 0.5×10^{-6} eq./g. Larger amounts of *o*-iodosobenzoate caused progressive decreases in loaf volume until about 1.1×10^{-6} eq./g. had been added, after which increasing amounts had no further influence. Levelling off of loaf volume occurred before all the sulfhydryl had been oxidized. On a weight basis *o*-iodosobenzoate was about 1.2 times as effective as bromate.

The process of ageing as shown by a decreased maximum response to *o*-iodosobenzoate was undoubtedly speeded up at higher tempera-

tures. Yet ageing at 40° C. for 1½ years was not accompanied by a significant decrease in sulfhydryl content, as measured by titration with this reagent.

Discussion

The determination of sulfhydryl groups in flour slurries by means of *o*-iodosobenzoate and radioactive iodine overcomes the difficulties encountered by conventional procedures. The errors observed in Table I are random errors, and can be lowered somewhat by taking the mean of duplicate samples. The unpredictable degree of adsorption of iodide ions which does occur to a small extent is probably the largest source of these errors.

The improving action of *o*-iodosobenzoate must occur through the oxidation of sulfhydryl groups, for it reacts quickly with flour slurries, while the reduced form *o*-iodobenzoate has no effect. The process of ageing in the second break flour as shown by decreased requirements for *o*-iodosobenzoate is undoubtedly accelerated at higher temperatures. This process is not accompanied by an oxidation of sulfhydryl groups yet the optimum requirements for *o*-iodosobenzoate fall off. It must be concluded that ageing involves some other factor which is important in baking. Hlynka and Bass (5) have shown that gluten in flour underwent a reaction with glucose during a five month storage period. This was suggested as evidence supporting the hypothesis that reducing carbohydrates in dough and gluten may act as cross-linking agents between protein chains to form a three-dimensional network. It may be that the best optimum dough quality is reached through a combination of such cross-linking agents. Thus, if the dough acquires some elastic properties through a protein-carbohydrate reaction less *o*-iodosobenaozte will be required to bring it to the optimum by disulfide linkages.

Acknowledgments

The authors are greatly indebted to Dr. J. W. T. Spinks for assistance in the radioactive portion of the project, both for equipment and technique. Thanks are also due to Mr. R. E. Teed for the baking results and the Quaker Oats Company, Saskatoon, Sask., for the commercial flour sample.

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QUANTITATIVE EXTENSOGRAPH STUDIES OF RELAXATION OF INTERNAL STRESSES IN NON-FERMENTING BROMATED AND UNBROMATED DOUGHS [1]

C. J. DEMPSTER,[2] I. HLYNKA,[3] and C. A. WINKLER [4]

ABSTRACT

Extensograms on spring wheat flour doughs were analyzed by reading the load in grams at an extension of 11 cm. on the kymograph paper. From these data, curves showing the relaxation of internal stresses in dough were derived. Relaxation is exponential and is characterized by a rate constant. In untreated doughs, no apparent change in rate of relaxation of internal stresses occurred with increasing reaction time. On the other hand, in bromated doughs the rate of relaxation decreased with increasing reaction time. The bromate reaction, however, is latent. The bromate reaction appears to be zero order; for a given bromate concentration, the rate of relaxation decreased linearly with increasing reaction time. Moreover, the higher the bromate concentration in dough, the more rapid is the bromate reaction.

By analogy with polymer concepts, it is postulated that the observed behavior of flour dough is determined by an internal network structure, the cross links of which are labile bonds between polar groups of adjacent molecules. The bromate reaction progressively introduces relatively non-labile cross links in the postulated network. Bromate, it is postulated, creates potentially reactive groups in certain molecules of the network. These groups interact to form cross links between adjacent molecules only when brought together when the dough is worked.

Fundamental rheological studies of flour dough (3, 4, 5, 7, 9) have been limited and have not led to hypotheses describing the structure

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and behavior of dough in chemical terms. The present investigation constitutes an attempt, through studies of the changes produced in physical dough properties, to elucidate the chemistry of the improving action of potassium bromate in flour dough, and more broadly to relate observed physical properties to the molecular structure of dough.

Measurements of changes in gross physical properties of flour dough were made with a Brabender Extensograph, but the method used to analyze the extensograms differs from any method previously used. None of the previous extensogram measurements (height, length, ratio, area, 6) have yielded data which could be used in a fundamental description of dough behavior. The extensogram measurement used in the present investigation is that of the load supported by each dough (i.e., resistance to extension) at some particular value of constant sample extension. This method of analysis is somewhat analogous to a measurement which has been previously employed (1) in fundamental studies of rubber and other elastomers. The measurement is essentially one of stress, since, at constant sample deformation, different doughs exhibit virtually equal cross-sectional areas.

This method of extensogram analysis has been employed in a study of "relaxation of internal stresses" in flour doughs. It is known (6) that with increasing rest period between shaping (i.e., rounding and rolling a dough on the extensograph) and subsequent stretching, the resistance a dough offers to extension decreases rapidly from very high values, while the extensibility increases. These changes in extensograph dough properties with rest period after shaping will be referred to as "relaxation of internal stresses." A kinetic approach has been undertaken in a study of the rates of relaxation of internal stresses in untreated and bromated doughs. A study of the rate at which changes in these physical properties of bromated doughs occur was utilized to provide data on the basis of which an adequate conceptual system relating to molecular reactions and dough structure could be advanced.

Materials and Methods

The flour used in this study was milled from a composite sample of hard red spring wheats grading 1 and 2 Northern. The wheat was milled in an Allis-Chalmers experimental mill to an extraction of about 72%. The protein content of the flour was 13.3% on a 14% moisture basis. The flour absorption, determined with the farinograph, was 63%.

All doughs were made with 200 g. of flour, water equivalent to 63% absorption, and 1% of sodium chloride. Doughs containing no salt would have been preferred but are too sticky for extensograph studies. Doughs were mixed for three minutes in a specially-designed mixer.

Dough ingredients were temperature-conditioned over night so that the doughs were taken from the mixer at a temperature of 30°C. When required, potassium bromate in solution was added in specified amounts.

A Brabender Extensograph was employed to study changes in the physical properties of bromated and unbromated doughs. Settings were adjusted so that the vertical paper units read directly in grams. The various steps in the extensograph testing of doughs in this study were as follows. Flour doughs were mixed from 200-g. samples of flour. From each such parent dough, two 150-g. test pieces were weighed out. The doughs were then set aside, without further handling, for a given length of time referred to as the "reaction time." During the reaction time, the test doughs were kept in individual crocks in a cabinet maintained at 30°C. and 85% relative humidity.

At the end of any desired reaction time, the doughs were subjected to the customary treatment in the extensograph. Each dough was placed in the rounder and the base plate was given exactly 20 turns. The dough ball was then passed once through the roller to obtain a test piece of the required dimensions. These operations—the rounding and rolling of the dough sample—will be referred to as "shaping."

After shaping, the doughs were clamped in the dough holders and returned to the cabinet. Varying rest periods, from 5 minutes to 180 minutes or more, were then allowed between shaping and stretching of a test sample. Each dough was stretched only once and then discarded.

Extensograms were analysed by recording the load supported by each dough at an extension of 11 cm. on the kymograph paper. This extensogram extension, as a basis for the method of measurement, was chosen arbitrarily. It is later shown, however, that the selection of a particular value of constant sample deformation does not significantly influence the subsequent interpretation of the derived data. It should be noted that to obtain loads at exactly constant deformation a correction should be made for the small movement of the arm supporting the dough. But this correction is systematic and relatively small. For example, as the load supported by the dough at an extension of 11 cm. on the extensogram increases from 300 g. to 900 g. the correction increases by about 10 g. The error introduced by neglecting the correction is usually much smaller and well within the experimental error of the method as a whole.

In order to illustrate the method and certain basic concepts which are fundamental to a clear understanding of the experimental results to be presented, a specimen investigation will now be described.

Specimen Investigation

The present investigation deals with the effects of three variables on extensograph dough properties: (1) Reaction time, i.e., the time between mixing and subsequent shaping of doughs into the required cylindrical test piece. (2) Rest period, i.e., the time between shaping and subsequent stretching of a dough sample on the extensograph. (3) Bromate concentration.

The effects of rest period and bromate concentration on the extensograph properties of doughs allowed no reaction time were investigated as follows. Eight 150-g. unbromated doughs were prepared and were shaped on the extensograph immediately after mixing. Each dough was allowed a different rest period before being stretched on the extensograph. A similar procedure was followed for nine 150-g. doughs containing 5 mg.% bromate. With increasing rest period, the extensograms showed a gradual decrease in height and a coincident increase in length. Such a change in physical dough properties with rest period after working is quite well known and will be referred to as relaxation of internal stresses in the dough or simply relaxation. The load supported by each dough at 11 cm. kymograph extension was read from the extensograms. A plot of load against rest period for both the control and bromated doughs yields the two curves shown in the left-hand portion of Fig. 1. This type of relaxation should be clearly distinguished from relaxation of stretched doughs held at constant extension, studied by Schofield and Scott Blair (8).

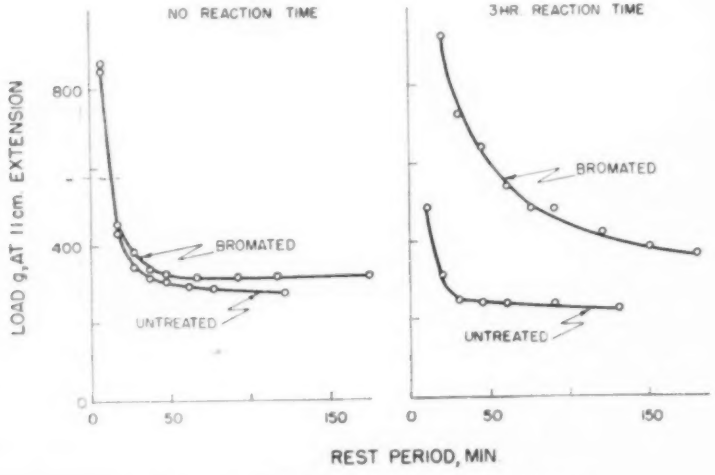

Fig. 1. Effect of reaction time on relaxation of internal stresses in untreated and bromated doughs.

These curves for untreated and bromated doughs allowed no reaction time show a fairly marked change in extensogram load with rest period. The rate of change of extensogram load with rest period indicates the rate at which stresses set up in the dough by the shaping operation are dissipated. When bromate is present in flour doughs, its effect on extensograph dough properties is scarcely apparent when no reaction time is allowed. For both bromated and untreated doughs, allowed no reaction time, the load supported by the doughs in the extensograph test decreased at about the same rate with increasing rest period, the curves being virtually superimposed for short rest periods. With extended rest period for bromated doughs, the load decreased to a stable value; while for untreated doughs, the load decreased almost linearly with time.

The effect of reaction time on the extensograph properties of untreated and bromated doughs was investigated as follows. Seven 150-g. untreated doughs were prepared and allowed a 3-hour reaction time between mixing and shaping on the extensograph. Each dough was then allowed a different rest period before being stretched on the extensograph. A similar procedure was followed for nine 150-g. 5 mg.% bromated doughs. The data derived from these extensograms are shown in the two curves in the right-hand portion of Fig. 1. These curves show that after a reaction time has been allowed there is a marked difference in the rate of relaxation of internal stresses in bromated and untreated doughs. Relaxation takes place much more slowly in the bromated doughs.

The curves in Fig. 1 depicting relaxation of internal stresses in unbromated doughs are quite similar for both 0- and 3-hour reaction times. Reaction time does not appear to alter the rate of relaxation in untreated doughs although the curve for the 3-hour reaction time does appear to fall slightly lower on the load axis than the curve for 0 reaction time. On the other hand the curves for the bromated doughs allowed 0 and 3 hours reaction time show that increasing reaction time produces a decrease in the rate of relaxation of internal stresses.

The relaxation curves of Fig. 1 illustrate two very important characteristics of the bromate reaction in dough. These are:

(a) For the bromate reaction to take place, as indicated by changes in the physical properties of dough, a reaction time must be allowed between the mixing and shaping operations.

(b) For the bromate reaction to be reflected in physical properties of doughs tested on the extensograph, work must be done on the dough as in the shaping operation, after the reaction time has elapsed.

General Results

More detailed experiments than those described above were carried out to determine the effect of reaction time on the relaxation of internal stresses in untreated control doughs and in doughs containing 3 mg.% and 5 mg.% bromate.

Relaxation of Internal Stresses in Untreated Doughs. Five series of unbromated control doughs were prepared and allowed reaction times of 0, 1, 2, 3, and 4 hours, respectively, between the mixing and shaping operations. At the end of a given reaction time, the doughs were shaped on the extensograph and then allowed various rest periods before being stretched.

Representative data obtained by analyzing these extensograms are given in Fig. 2 where load is plotted against rest period for reaction

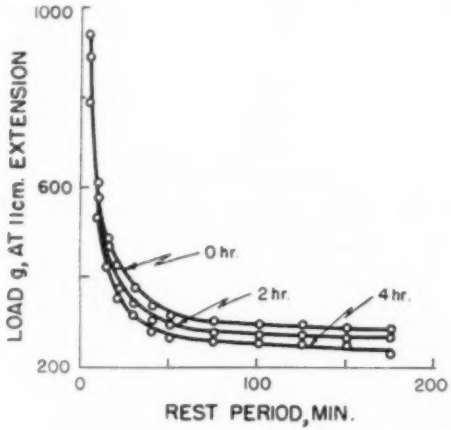

FIG. 2. Effect of reaction time on relaxation of internal stresses in untreated doughs.

times of 0, 2, and 4 hours. The data for a reaction time of 1 hour were intermediate between that of 0- and 2-hour reaction times; while the 3-hour reaction time data were intermediate between that of the 2- and 4-hour reaction times. With increasing rest period between shaping and subsequent stretching, the load supported by a dough decreased quite rapidly at first from very high values, and then shows a slow almost linear decrease.

The relaxation curves of Fig. 2 indicate that increasing reaction time produces a gradual downward displacement of the curve. However, the curves do not appear to exhibit any change in shape as reaction time is increased. The rate of relaxation of internal stresses in un-

bromated flour doughs appears to be independent of the reaction time allowed between mixing and shaping the doughs.

Relaxation of Internal Stresses in Bromated Doughs. An experiment was carried out to determine the effect of reaction time on the relaxation of internal stresses in doughs containing 3 mg.% and 5 mg.% bromate. Doughs containing 3 mg.% bromate were allowed reaction times of 0, 1, 2, 3, and 4 hours between mixing and shaping, while doughs containing 5 mg.% bromate were allowed reaction times of 0, 1, 2, and 3 hours. At the end of the prescribed reaction time, the doughs were shaped, allowed various rest periods and then stretched on the extensograph. The data obtained by analyzing these extensograms are recorded in Fig. 3 where load is plotted against rest period for each reaction time and each bromate concentration.

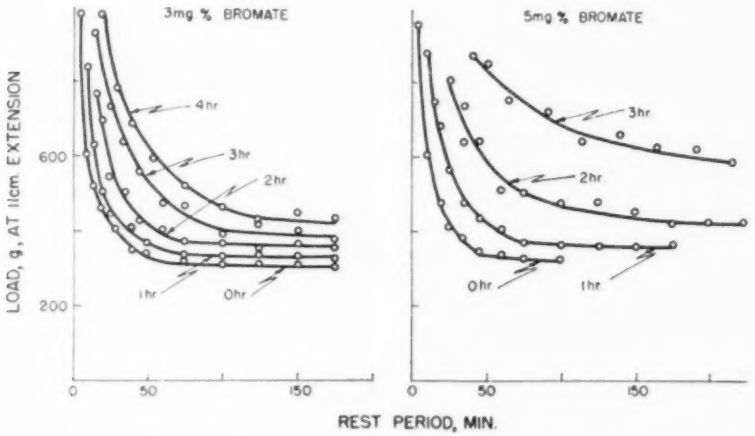

FIG. 3. Effect of reaction time on relaxation of internal stresses in doughs containing 3 mg.% and 5 mg.% bromate.

Figure 3 illustrates the pronounced effect produced by bromate, with increasing reaction time and subsequent shaping, on the physical properties of flour doughs. The relaxation curves of Fig. 3 show that with increasing reaction time for a given bromate concentration, the rate of decrease of extensogram load becomes progressively slower. This effect of bromate on physical dough properties with increasing reaction time is more pronounced the greater the bromate concentration in the dough. Further, as previously noted, the presence of bromate in dough prevented the slow linear decrease of load with rest period observed in untreated doughs. With increasing rest period in bromated dough, the load decreases to a stable value which depends upon the bromate concentration and the reaction time. For a given

bromate concentration, the final stable load value was higher the longer the reaction time. In addition, the increase in the final load value with increasing reaction time was more rapid at the higher concentration of bromate. It is especially important to note that the effect of bromate is apparent only if the doughs are shaped following the reaction time. This point has also been noted by Smith and Andrews in concurrent studies (8).

An interesting observation with respect to the relaxation of internal stresses in doughs can be made by comparing the curves of Fig. 2 and Fig. 3. Figure 2 shows that for untreated control doughs, the load supported by a dough has passed through the stage of rapid decrease in a rest period of 45 minutes. This corresponds to the rest period generally employed in routine dough testing with the extensograph. The

TABLE I

FINAL LOAD AND RELAXATION CONSTANTS FOR BROMATED DOUGHS

Reaction Time	Bromate Concentration	Final Load	Relaxation Constant
hr.	*mg./l.*	*g.*	*k.*
0	3	310	0.053
1	3	330	0.044
2	3	350	0.042
3	3	400	0.036
4	3	420	0.032
0	5	320	0.057
1	5	370	0.037
2	5	430	0.028
3	5	500[1]	0.010

[1] Final load estimated from the relaxation data by extrapolation since a stable value had not been attained within the rest periods allowed.

physical properties of untreated doughs, regardless of reaction time, become more or less stabilized during a 45-minute rest period, comparatively little change occurring with further rest period. Figure 3 shows that, for bromated doughs, depending upon the concentration and the reaction time, considerable change in physical dough properties may occur after a rest period of 45 minutes.

The shape of the relaxation curves of Fig. 3 suggested that for bromated doughs the decrease of load with increasing rest period obeys an exponential decay law although the decay did not continue to zero load. Calculations were made to determine whether the decay of load with rest period was actually exponential. For each relaxation curve of Fig. 3, the load values were "corrected" by subtracting the final load at the limit of decay. The values of the final load used in the correction for each reaction time are recorded in Table I. Logarithmic plots

of the "corrected" loads against rest period for each bromate concentration and each reaction time are shown in Fig. 4. The curves are linear over a considerable range of rest periods for each bromate concentration at each reaction time.

The linearity indicates that the decay of load with rest period is exponential, i.e., it obeys an equation of the Maxwellian type:

$$L = L_0 e^{-kt}, \tag{1}$$

where L = load at any time, t,
L_0 = load at time $t = 0$,
k = rate of relaxation or relaxation constant.

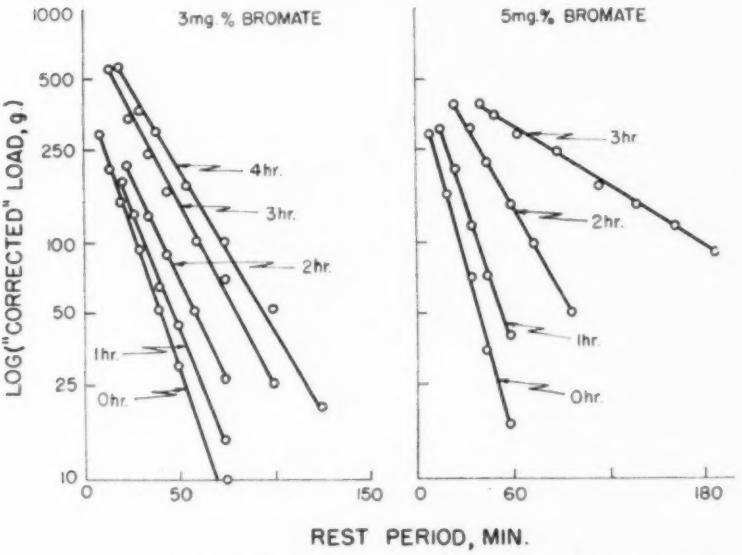

FIG. 4. Exponential relationship between load and rest period in relaxing doughs.

The fact that the relaxation of internal stresses in dough can be characterized by an exponential decay law provides a convenient quantitative means of following the progressive changes in physical dough properties produced by the reaction of bromate. Each relaxation curve of Fig. 3 can be characterized by a relaxation constant, k, as in expression (1). The relaxation constant for a given relaxation curve can be obtained simply by calculating the slope of the corresponding logarithmic curve in Fig. 4, and multiplying this figure by the factor -2.303 since common logarithms were used. The relaxation con-

stants were calculated for each bromate concentration and each reaction time. These values are recorded in Table I.

A plot of relaxation constant against reaction time for both the 3 mg.% and the 5 mg.% bromated doughs is given in Fig. 5 from which it appears that the rate of relaxation decreased linearly with increasing reaction time, and that the decrease is more rapid at the higher bromate concentration.

Analysis of Extensogram Data at Various Sample Dimensions. The data presented above were derived from extensograms by reading off the load supported by the various doughs at a constant, arbitrarily chosen, extensogram extension of 11 cm. This differs from the procedure generally employed in analyzing extensograms where the maxi-

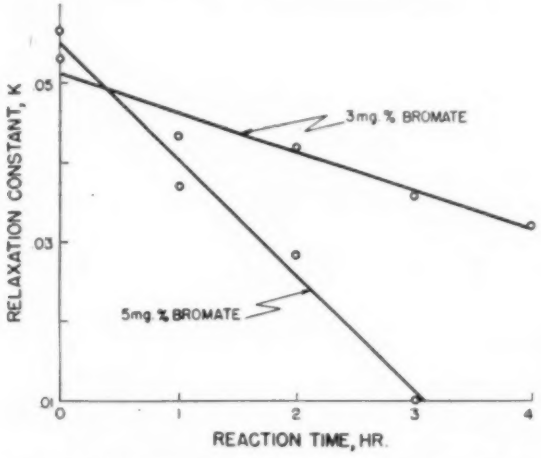

FIG. 5. Relation between rate constant for relaxation and reaction time.

mum load (i.e., maximum height) is taken. It was obviously a point of interest to determine whether the two methods of analysis yielded essentially the same information about the relaxation of internal stresses in dough.

The extensograms obtained in this study were therefore reviewed and the loads were recorded at extensogram extensions of 3, 7, and 11 cm. together with the maximum load. The maximum load, of course, does not occur at a constant extension. For a single experiment, i.e., for the extensograms obtained for a single reaction time, these data give rise to four curves when load is plotted against rest period—one curve for each of the three values of constant extension and one for the load at the maximum. The extensograms of a large number of experiments gave results, by these different methods of analysis, which were

essentially the same from one experiment to another. Some typical values are plotted in the left-hand portion of Fig. 6.

These relaxation curves of Fig. 6 all show the same general characteristics, i.e., a more or less rapid initial decrease of load with rest period to a stable value at longer times. The curves are, of course, displaced from one another along the y- or load axis according to the elongation at which the load was measured.

Correction of the data of each of the relaxation curves of Fig. 6 was made in the manner previously described (i.e., by subtracting the final load value for a given curve from each point on that curve), and a logarithmic plot of the corrected load against rest period was made for each value of constant deformation. These log. plots are shown in the right-hand portion of Fig. 6.

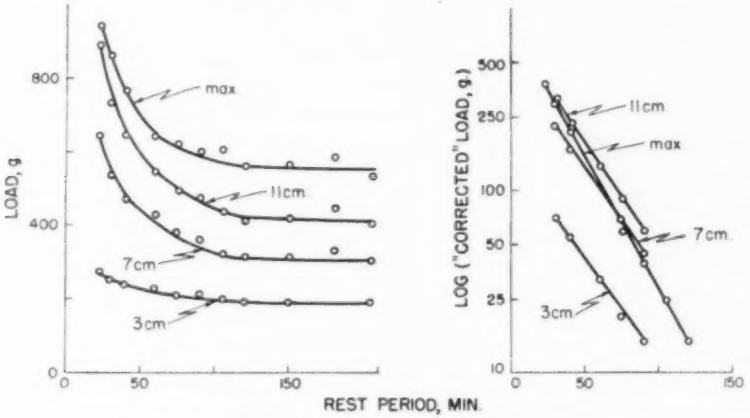

FIG. 6. Analysis of relationship between load supported by relaxing doughs and rest period at various extensogram lengths.

The logarithmic plot of the corrected load against rest period is linear over a considerable range of rest periods for each of the different elongations. This linearity of the logarithmic plot confirms, as noted previously, the exponential decay of load with rest period. Of further interest is the fact that the curves obtained for three different values of constant elongation were virtually parallel. The data for the maximum extensogram load also gave a linear log. plot, but this curve was not parallel to the other three curves.

The analysis of extensograms, using the load at the curve maximum, does not seem to yield exactly the same information about the relaxation of internal stresses in dough as does the analysis using the load at a constant sample deformation. However, the latter would seem to be preferred as a basis for the analysis of extensograms since the in-

formation obtained about the relaxation of internal stresses in dough is essentially the same regardless of the particular value of constant sample deformation.

Discussion

The significant observations made during this study may be summarized as follows: Internal stresses set up in unbromated doughs by shaping relax at a rate that is independent of the reaction time between mixing and shaping. But in corresponding bromated doughs, the rate of relaxation of internal stresses decreases with increasing reaction time and with increasing concentration of bromate. Moreover, the reaction of bromate in flour dough is latent; changes in physical dough properties due to the reaction of bromate appear only when the dough is worked or shaped after a time of reaction.

Explanations of these effects may be sought by analogy with concepts developed in studies of rubber and other natural and synthetic elastomers. Since dough is partially elastic, it may be postulated that it contains flexible, long-chain molecules (presumably protein) with some cross links between neighboring molecules creating a three-dimensional or network structure. The cross links may be primary covalent bonds but are more probably points of strong intermolecular or secondary valence forces between polar groups of adjacent molecules. Sections of the long molecules between cross links assume randomly kinked or crumpled configurations. But the structure is dynamic so that the shape and degree of kinking in individual molecular segments change readily.

In a rested dough, the lengths of the molecular segments between the cross links of the postulated network structure are considered to be normally distributed about a "most probable length"; that is, the segments are randomly kinked and randomly oriented with respect to each other. A certain minimum number of the polar groups of adjacent molecules are considered to be involved in intermolecular cross linkages. These cross links are labile: cross links between adjacent molecules are not always in the same position but the average number of bonds remains the same. The system is characterized by a certain equilibrium energy, the magnitude of which determines the number of cross links in the postulated network structure.

When a rested dough is shaped, by the comparatively mild manipulations involved in rounding and rolling, it is postulated that the mean length of the molecular segments is increased by mechanical unkinking and that previously non-bonded polar groups in adjacent molecules are brought into juxtaposition. Intermolecular forces between these groups establish additional cross links in the network. Internal stresses are thus set up in dough by working and a considerable force is required to stretch the dough.

The work done on the dough by shaping together with the energy available from bond formation will result in an increase in the energy of the system. When the thermal kinetic energy of the molecular segments is greater than the energy of the polar bond, then rupture of some of the cross links formed during shaping will occur. Bond rupture will continue until the average kinetic energy of the segments is of the order of the bond energy. An equilibrium will then exist between number of bonds breaking and re-forming. Thus, during a rest period following shaping, the thermal kinetic motions of the molecular segments will cause the network to return to the equilibrium state. As the internal stresses thus relax, the load required to stretch a dough will decrease. The rate of relaxation is indicative of the rate at which the postulated network structure returns from the partly oriented, highly cross-linked arrangement induced by working the dough to the equilibrium arrangement. The rate of relaxation of internal stresses is determined by the extent of cross linking of the network structure. Changes in the rate of relaxation, therefore, indicate changes in the degree of cross linking.

The curves of Fig. 2 showing the relaxation of internal stresses in untreated flour doughs indicate that the rate of relaxation did not change appreciably as the reaction time was increased from 0 to 4 hours. This is interpreted as an indication that no change in the postulated polymeric network structure of the dough occurred. For each reaction time, the initial exponential decay of load is succeeded by a slight linear decrease of load with further rest period. This behavior is not believed due to chemical change, such as proteolysis. For were proteolysis taking place, a progressive change in the dough structure due to the degradation of the protein molecules in the network should occur. This would be reflected in changes in the rate of relaxation of internal stresses as reaction time was increased. The linear decrease of load with rest period may be due to a progressive softening of the dough with time, a behavior which has been observed by some workers (2, 3).

The curves of Fig. 3 show that the incorporation of potassium bromate into flour doughs produces a gradual but fairly marked change with increasing reaction time in the rate of relaxation of internal stresses. These changes are dependent upon the bromate concentration. By analogy with elastomer chemistry, it is postulated that the bromate effect results from the creation of non-labile cross links in the network structure of the dough. These cross links would be expected to damp the kinetic motion of the molecular segments, thus decreasing the rate of return to a random arrangement, i.e., the rate of decay of internal stress. Moreover, the additional cross links would be expected to change the properties of the network structure after complete re-

laxation. This is manifest, as the relaxation curves show, by the greater final load required to stretch the dough.

The curves of Fig. 5 show that for a given bromate concentration in dough, the rate of relaxation of internal stresses decreased linearly with increasing reaction time. The linearity of the change in rate of relaxation with reaction time indicates that the reaction of bromate in dough takes place at a steady rate, i.e., reaction is zero order. The bromate reaction depends upon the bromate concentration since a more rapid change in relaxation rate occurs at the higher bromate concentration.

It is now necessary to account for the fact that the bromate effect requires a reaction time and is shown only after shaping. The latter observation suggests that the postulated cross links due to the bromate reaction are formed only after shaping. A possible explanation is that during the reaction time potential points of cross linking are activated by bromate. These active centers are created in a time-dependent reaction to an extent which is determined by the bromate concentration. However, the reaction of these active centers does not take place at any appreciable rate in a resting dough, owing to the lack of free migration of molecules through the dough. Consequently, contact does not take place between the reactive groups in adjacent molecules. Conditions for a zero order reaction prevail; the shaping of the dough serves to bring these reactive centers together so that cross links may actually form.

The above conceptual system is postulated to account for the observations recorded in this paper. It may well serve to indicate further lines of investigation designed to elaborate more adequately the hypothesis of the chemistry of the bromate reaction, and of dough structure in general. Study of the kinetics of the molecular reactions is only a first step; investigations must then be carried out to identify the actual groups responsible for the postulated cross linking. Proceeding thus in a step-wise fashion, it may be possible to synthesize a detailed picture of dough chemistry.

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GRAIN STORAGE STUDIES. X. THE INFLUENCE OF AERATION, TIME, AND MOISTURE CONTENT ON FAT ACIDITY, NONREDUCING SUGARS, AND MOLD FLORA OF STORED YELLOW CORN [1]

R. A. Bottomley,[2] C. M. Christensen,[3] and W. F. Geddes [4]

ABSTRACT

In aerated samples of No. 1 yellow dent corn stored at 30°C. at four moisture levels between 19 and 31%, the mold count and fat acidity generally increased while the viability and nonreducing sugars decreased with increasing moisture content and time of storage. However, the numbers and kinds of molds varied rather widely between duplicate trials. Different species predominated at various intervals of storage and moisture contents. Decreases in nonreducing sugars paralleled mold count more closely than did the increases in fat acidity. The growth of *Aspergillus glaucus* appeared to cause a rapid loss in nonreducing sugars and little increase in fat acidity while the growth of *A. flavus, A. candidus, Penicillium* sp., and *Fusarium* sp. was often associated with a marked increase in fat acidity.

In nonaerated samples, there was relatively little change in fat acidity or nonreducing sugars but there was a decrease in viability. Certain molds, especially *Cephalosporium* sp., continued to increase in the samples stored at about 31% moisture even when the oxygen content of the interseed atmosphere had fallen to almost zero, and the carbon dioxide concentration exceeded 30%. Under these conditions, fat acidity remained relatively constant and was a poor measure of grain damage. These observations indicate that spoilage of high-moisture grain cannot be completely prevented by storing it under hermetically-sealed conditions.

Recently, Bottomley *et al.* reported the effects of variations in temperature and in oxygen concentration of the interseed atmosphere

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upon mold counts, viability, and several biochemical properties of yellow dent corn stored for 12 days at various moisture contents (2). In these experiments, the nonreducing sugar content appeared to be the most reliable single biochemical index of deterioration. The increase in mold count was closely correlated with the decrease in nonreducing sugars in the continuously aerated samples but not in those stored hermetically. Fat acidity was only of limited value as a criterion of storage deterioration and of mold development. Increases of mold count and fat acidity did not parallel one another with variations in the conditions of storage. The relation between these variables apparently was influenced by the kinds of molds which predominated and the length of time of their development.

These results, together with some general observations during the course of the storage trials, indicated that, on moist stored seed, there might be a succession of different molds, each predominating for a time and each producing somewhat different effects. If this were true, a determination of the numbers and kinds of molds present at the end of a given storage period, and the results of analyses for such constituents as nonreducing sugars and fat acidity, might fail to give a true picture of the extent of deterioration.

The present study was undertaken to follow the biochemical and microbiological changes that occur over short periods of time in moist stored corn under aerated and nonaerated conditions.

Materials and Methods

General Procedure. The procedures and apparatus used in the present studies were the same as those employed previously (2). Subsamples of a composite lot of No. 1 grade yellow dent corn of high viability (86–90%) were conditioned to moisture contents of about 19, 24, 27, and 31% (dry weight basis), which were in approximate equilibrium with relative humidities of 80, 90, 95, and 100%, respectively. Twelve 100-g. lots at each moisture content were stored in bottles immersed in a water bath at 30°C. Six of the bottles were kept sealed while the other six were aerated continuously with humidified air in equilibrium with the moisture contents of the respective samples. The air was passed through the samples at a rate which was just sufficient to prevent any appreciable accumulation of carbon dioxide in the interseed atmosphere. At two-day intervals, one sealed and one aerated sample at each moisture content were removed. At the end of the 12-day period the experiment was repeated with additional samples of corn from the same original well mixed lot.

The air in the sealed bottles was analyzed just before the samples were removed from the water bath for microbiological and biochemical

analysis. Fifty-five milliliters of interseed atmosphere were withdrawn through a tube almost reaching to the bottom of the bottle. The gas was slowly drawn into the tube under a slight vacuum and a small amount of air was admitted into the top of the bottle through a tube plugged with cotton. This operation did not obviate the possibility of slight contamination of the gas sample by incoming air, but since several of the gas samples so taken were free of oxygen, the sampling technic may be assumed to have been adequate to obtain a close approximation of the true interseed atmosphere.

The sealed and unsealed samples were air-dried immediately upon removal from the water bath and prepared for assay as previously described (2). Fat acidity and nonreducing sugars were determined by the methods outlined in Cereal Laboratory Methods (1). Moisture was determined on the air-dried samples by drying the ground samples for one hour in an air oven at 130°C. The total moisture was computed from the resulting values and from the moisture lost during air-drying.

Viability of the seed was determined by surface disinfecting 50 seeds in a 1.5% solution of sodium hypochlorite for two minutes, then placing them on agar in petri dishes which were stored in the laboratory at 21°–23°C. for 5 to 7 days.

Determination of Numbers and Kinds of Molds. The number and kinds of molds were determined by a modification of the general procedure outlined by Christensen (3). Two grams of ground dried corn were suspended in sterile 0.3% agar solution, and subsequent dilutions made from which aliquots were taken for culturing.

Rather extensive studies of the dilution technic made before these tests were undertaken indicated that one of the principal sources of variation in mold count obtained from a given sample arises from unequal distribution of mold spores or other inoculum in the dilution bottle, when sterile water or sterile saline solution is used as the diluting or suspending medium. The variations between replicate 2-g. samples from a given lot of ground corn, and between operators, were relatively small compared with the variation between replicate portions of suspended material taken from the same dilution bottle. It was thought that a more viscous liquid than water might aid in maintaining a more uniform suspension of mold spores and of ground seed fragments. After various trials, a solution of 0.3% agar in distilled water was selected as the most suitable of those tested. It proved sufficiently viscous to keep spores and seed fragments uniformly suspended for several hours. For pipetting this, it was found desirable to cut off the ends of the 5 ml. pipettes used for the purpose, and to

recalibrate them so that they would deliver 5 ml. of the somewhat viscous suspension.

The composition of the agar medium upon which material such as this is cultured will have a profound effect upon the number and kinds of molds which develop, as has been shown by Christensen (3), James and Smith (4), and others. In this work, it was essential to use a medium that would give the largest count of those molds considered to be of greatest importance in the deterioration process. To select a suitable medium, a sample of moldy corn was cultured in various dilutions on different agar media. Typical results are shown in Table I.

TABLE I

NUMBER OF MOLD COLONIES FROM A SINGLE SAMPLE OF MOLDY CORN CULTURED ON ELEVEN DIFFERENT MEDIA

Medium No.	Composition	Molds per Gram
1	Standard Czapek-Dox	57,000
2	Czapek-Dox plus 0.02% boric acid	0
3	Malt-sucrose (20 g. malt ext., 60 g. sucrose per l.)	64,000
4	Malt-sucrose-boric acid (same as No. 3, with 0.02% boric acid)	51,000
5	Malt-salt-sucrose (20 g. malt ext., 60 g. sodium chloride, 60 g. sucrose per l.)	82,000
6	Malt-salt-sucrose-boric acid (same as No. 5, with 0.02% boric acid)	43,000
7	Malt-salt-boric acid (20 g. malt ext., 60 g. sodium chloride, 0.2 g. boric acid per l.)	128,000
8	Modified Smith-Humfeld (1 g. $NaNO_3$, 1 g. KH_2PO_4, 20 g. sucrose per l.)	112,000
9	Modified Smith-Humfeld-salt-boric acid (same as 8, with 60 g. sodium chloride, 0.2 g. boric acid per l.)	72,000
10	Malt-salt (20 g. malt extract, 60 g. sodium chloride per l.)	130,000
11	Czapek-Dox-salt (standard C-D plus 60 g. sodium chloride per l.)	90,000

Another illustration of the influence of the composition of the medium upon the mold count is shown in Table II in which the number of colonies of five different molds, which appeared on three different media, are recorded.

It is obvious from both tables that the standard Czapek-Dox medium, which is a widely used and valuable medium in many kinds of mycological work, is not suitable for determining the mold count of these samples. It not only gave one of the lowest total mold counts, but, as indicated in Table II, *Aspergillus glaucus* did not appear at all, whereas the other media indicated that this fungus was present in considerable numbers.

As a result of these preliminary tests, either malt-salt or malt-salt-boric acid media were used in determining the mold counts.

The essential steps in making the dilutions and cultures were as follows: For the original suspension of ground corn 53 ml. of hot, 0.3% agar solution and about 20 g. of quartz sand were placed in a 4 oz. screw-capped medicine bottle. After autoclaving and cooling to room temperature, each bottle contained 48.5 ml. of solution. The addition of 2.0 g. of ground corn gave a total volume of 50 ml., and a dilution of 1:25. This bottle was shaken on a reciprocal shaker for 30 minutes at 240 strokes per minute. In each of the bottles in which subsequent dilutions were made, 48.5 ml. of the hot dilute agar solution were added. When autoclaved and cooled, the volume was 45 ml., so that the addition of 5 ml. of the suspension from the first bottle gave a further dilution of 1:10. A series of dilutions of each corn sample was made, and two of them were cultured. For culturing, 5 ml. of the suspension were added to 95 ml. of the culture medium cooled to 48°C., the bottle was upended several times slowly to mix the suspension with the culture

TABLE II

NUMBER OF COLONIES OF FIVE PREDOMINANT SPECIES OF STORAGE MOLDS OBTAINED BY CULTURING THE SAME SAMPLE OF MOLDY CORN ON THREE DIFFERENT MEDIA

Medium	Total Number of Colonies on 5 Replicate Plates				
	A. glaucus	*A. flavus*	*A. tamarii*	*A. candidus*	*Penicillium*
Czapek-Dox	0	65	42	0	16
Malt-salt-sucrose	57	55	46	0	21
Malt-salt-boric acid	58	57	42	49	43

medium (rapid shaking of the relatively cool agar results in the formation of foam which remains on the surface of the agar in culture dishes and interferes with detection of mold colonies) and poured into five sterile Petri dishes. The dishes were stored under glass jars in the laboratory at 21° to 23°C. until the colonies were counted.

After the plates had incubated two to three days, each plate was placed upside down, unopened, on the stage of a stereoscopic microscope and examined by transmitted light. The magnification used was × 10 to × 30. In this preliminary examination it was found desirable to adjust the mirror of the microscope so that one side of the field was rather brightly lighted, the other side rather dimly lighted. The location of colonies was marked on the bottom of the dish with a wax pencil. Thus the colonies which appeared on successive days could be accurately counted. James and Smith (4) objected to the use of a microscope in counting mold colonies in culture dishes. They suggested that one might, by using a microscope, count secondary

colonies that had arisen from spores produced by some of the more rapidly growing and sporulating fungi in the culture dish. In our tests, the fastest growing and sporulating molds never produced spores in less than five days, and secondary colonies did not appear before seven to eight days. Thus, examination after two, three, or four days would not reveal secondary colonies. Colonies often arise within a fraction of a mm. of one another, even when there are only 30 to 50 colonies per plate. Also, colonies may be separated vertically in the agar, but not horizontally. In these cases, the individual colonies can be detected with the stereoscopic microscope, but not with the naked eye. For some samples, examination of the culture dishes two or three days old with the stereoscopic microscope was not only a valid, but an essential aid in determining mold count.

The mold counts in the present study represent the total number of colonies that appeared on five plates multiplied by the dilution factor. In most cases, counts were made from plates that had 30 to 50 colonies.

Results

The results for the samples of the two trials that were continuously aerated are given in Table III and those for the samples of the two trials that were stored in sealed containers are given in Table IV. The corn used in these tests was thoroughly mixed and was supposedly a uniform lot. For each two-day sample, a single jar of corn was used and the duplicate tests were made after the completion of individual tests on the entire series. The numbers and kinds of molds as well as the results of the biochemical determinations frequently varied so widely in the two trials that average values would be meaningless, and the individual results have therefore been recorded.

Aerated Samples. The mold count and fat acidity of the aerated samples, in general, increased as the moisture increased while the viability and nonreducing sugars decreased. At any given relative humidity or moisture content, the extent of these changes increased with time of storage. In samples stored at 19.0% moisture (80% relative humidity), the mold count increased from initial values of 10,000–14,000 per g. to over 30,000 after 12 days. The viability of the corn fell from an initial value of 85% to 53% during the trial but there was little change in fat acidity or nonreducing sugar content. In contrast, the corn stored at the highest moisture level (31.9% in equilibrium with 100% relative humidity), the mold count in the first test increased from an initial value of 44,000 to 32,000,000,000 per g. after 12 days' storage; the viability fell to zero, the fat acidity increased approximately four-fold and the nonreducing sugars decreased to one-seventh of their original value. In the second trial at 31.1% moisture,

TABLE III

Changes in Biochemical Properties and Mold Flora of Corn Stored Twelve Days with Continuous Aeration, at Four Moisture Contents

Days Stored	Test No.	Fat Acidity[1]	Nonreducing Sugars[2]	Germin. (%)	Mold Count (per g.)	Per Cent of Total Made Up by Each Mold Species						
						A. ustus	*A. candidus*	*A. flavus*	*A. tamarii*	*Penicillium* sp.	*Mucor* sp.	*Fusarium* sp.
80% Relative humidity. Initial moisture: Test 1, 19.0%; test 2, 19.3%. Final moisture, 18.1–19.9%.												
0	1	18.9	126.4		1,600	0	0	10	0	90	0	0
	2	19.1	156.2	85	10,700	0	0	5	0	95	0	0
2	1	18.8	105.8		2,900	5	0	5	0	90	0	0
	2	23.2	138.1	68	1,500	0	0	0	0	100	0	0
4	1	21.4	109.0		3,200	5	0	5	0	90	0	0
	2	21.2	142.1	84	3,500	20	0	0	0	80	0	0
6	1	21.2	107.9		80,000	0	0	40	40	15	5	0
	2	21.2	154.2	95	36,500	0	0	5	0	95	0	0
8	1	22.7	104.1		140,000	20	0	30	30	20	0	0
	2	24.4	147.9	54	190,000	0	0	80	0	5	15	0
10	1	21.7	105.7		240,000	20	5	5	60	5	5	0
	2	41.0	146.4	68	300,000	0	0	50	0	50	0	0
12	1	20.5	96.5		305,000	65	5	5	20	5	0	0
	2	25.2	147.8	53	350,000	0	0	60	0	0	0	40
90% Relative humidity. Initial moisture: Test 1, 24.0%; test 2, 23.5%. Final moisture, 23.4–24.7%.												
0	1	22.4	99.3		89,000	0	0	5	0	95	0	0
	2	21.7	157.2	84	3,500	0	0	20	0	80	0	0
2	1	20.4	101.1		54,000	95	0	0	0	5	0	0
	2	17.0	140.4	87	25,000	0	0	5	0	95	0	0
4	1	18.6	83.3		1,920,000	95	0	0	0	5	0	0
	2	19.5	137.3	63	175,000	100	0	0	0	0	0	0
6	1	21.7	64.0		1,200,000	95	0	0	0	0	5	0
	2	29.1	114.1	30.5	385,000	75	0	5	0	20	0	0
8	1	32.3	62.1		3,200,000	85	0	5	0	10	0	0
	2	56.3	101.6	19.0	3,500,000	15	0	20	0	0	65	0
10	1	36.2	70.6		4,700,000	80	0	5	5	5	5	0
	2	83.0	75.8	6.5	3,000,000	50	20	5	0	25	0	0
12	1	49.9	56.8		5,550,000	75	0	5	0	20	0	0
	2	96.7	61.4	3.0	7,750,000	20	5	0	0	5	0	70

[1] Expressed as mg. of potassium hydroxide per 100 g. corn, dry basis.
[2] Expressed as mg. of sucrose per 10 g. corn, dry basis.

TABLE III—(*Continued*)

Days Stored	Test No.	Fat Acidity[1]	Nonreducing Sugars[2]	Germin. (%)	Mold Count (*per g.*)	Per Cent of Total Made Up by Each Mold Species						
						A. glaucus	*A. candidus*	*A. flavus*	*A. tamarii*	*Penicillium* sp.	*Mucor* sp.	*Fusarium* sp.
95% Relative humidity. Initial moisture: Test 1, 27.8%; test 2, 26.9%. Final moisture, 25.5–30.4%.												
0	1	23.3	108.7		16,500	5	0	0	0	95	0	0
	2	18.7	147.8	83.5	1,500	0	0	35	0	35	0	30
2	1	25.9	93.8		64,500	30	0	10	5	50	5	0
	2	20.8	122.3	76	190,000	5	0	0	0	0	0	95
4	1	37.9	93.5		2,500,000	50	0	10	5	30	5	0
	2	35.6	112.5	72.5	1,250,000	40	5	10	0	40	5	0
6	1	60.6	99.7	27.0	5,600,000	50	15	10	5	20	0	0
	2	70.6	98.6	32.0	680,000	30	10	30	0	10	15	5
8	1	104.8	56.9	15.0	15,800,000	10	15	65	5	5	0	0
	2	105.6	56.1	10.0	2,400,000	25	35	15	0	5	20	0
10	1	113.4	34.0	13.0	29,000,000	15	40	30	5	10	0	0
	2	125.6	52.5	9.9	9,350,000	0	0	15	0	0	0	85
12	1	146.8	24.9	7.0	51,000,000	10	50	25	0	15	0	0
	2	134.7	51.9	6.0	14,500,000	0	0	15	0	20	0	65
100% Relative humidity. Initial moisture: Test 1, 31.9%; test 2, 31.1%. Final moisture, 30.2–42.5%.												
0	1	28.1	84.3		44,000	0	0	10	0	90	0	0
	2	19.4	150.4	79	7,000	0	0	0	0	100	0	0
2	1	36.2	97.8		1,290,000	5	0	45	50	0	0	0
	2	27.9	129.9	54.5	225,000	5	0	5	0	75	15	0
4	1	155.6	57.1		26,500,000	0	0	50	50	0	0	0
	2	47.8	118.1	33.0	1,020,000	5	0	15	20	30	30	0
6	1	155.0	20.1	9.0	460,000,000	0	0	50	50	0	0	0
	2	78.6	86.0	16.0	5,000,000	0	0	85	0	5	5	5
8	1	96.5	27.1	4.0	1,950,000,000	0	0	40	60	0	0	0
	2	127.5	49.2	10.0	18,450,000	0	0	30	5	0	10	55
10	1	116.2	18.4	2.0	15,500,000,000	0	0	50	50	0	0	0
	2	127.3	29.0	14.0	59,000,000	0	0	25	5	10	0	60
12	1	106.4	12.8	0.0	32,000,000,000	5	0	50	45	0	0	0
	2	124.1	25.6	2.5	61,000,000	0	0	20	10	10	0	60

[1] Expressed as mg. of potassium hydroxide per 100 g. corn, dry basis.
[2] Expressed as mg. of sucrose per 10 g. corn, dry basis.

TABLE IV

CHANGES IN BIOCHEMICAL PROPERTIES AND MOLD FLORA OF CORN STORED TWELVE DAYS IN SEALED CONTAINERS AT FOUR MOISTURE CONTENTS

Days Stored	Test No.	Fat Acidity[1]	Nonreducing Sugars[2]	Germin. (%)	CO_2 (%)	O_2 (%)	Mold Count (*per g.*)
80% Relative humidity. Initial moisture: 19.2%; final moisture: 17.8–19.2%.							
0	1	22.7	132.0	88.0	0.01	20.95	580,000
	2	21.3	151.8	81.0	0.01	20.95	—
2	1	22.2	131.0	86.0	—	—	200,000
	2	20.0	146.6	75.0	—	—	11,500
4	1	23.8	132.4	88.5	0.89	20.63	74,000
	2	22.0	143.7	69.5	0.89	20.64	60,000
6	1	21.7	135.9	63.5	1.53	20.67	200,000
	2	23.8	140.2	79.0	1.00	20.39	64,000
8	1	24.4	129.4	78.0	2.08	19.06	23,000
	2	21.6	140.9	59.5	1.95	19.19	12,500
10	1	24.8	149.2	70.0	2.69	18.67	22,500
	2	25.2	137.7	66.0	2.69	18.49	29,000
12	1	23.9	129.8	55.5	3.86	17.55	260,000
	2	22.4	133.3	64.0	2.63	17.45	89,000
90% Relative humidity. Initial moisture: 23.6%; final moisture: 22.4–24.5%.							
0	1	18.5	145.8	88.0	0.01	20.95	200,000
	2	23.5	133.9	73.5	0.01	20.95	51,000
2	1	18.1	132.4	84.5	—	—	29,500
	2	18.8	123.7	80.0	—	—	19,500
4	1	25.5	120.0	86.0	21.06	1.01	410,000
	2	20.3	123.4	77.0	19.03	2.97	36,000
6	1	24.7	126.2	81.0	22.45	2.26	190,000
	2	25.0	118.5	53.0	20.63	3.14	610,000
8	1	25.7	119.7	70.0	23.25	1.36	360,000
	2	36.7	99.1	48.5	21.23	2.74	435,000
10	1	27.4	90.2	62.5	23.78	1.04	1,150,000
	2	25.7	84.2	58.0	23.63	1.33	435,000
12	1	26.8	103.0	56.5	23.47	1.45	1,500,000
	2	19.4	100.7	51.5	23.26	1.74	2,000,000

[1] Expressed as mg. of potassium hydroxide per 100 g. corn, dry basis.
[2] Expressed as mg. of sucrose per 10 g. corn, dry basis.

TABLE IV—(*Continued*)

Days Stored	Test No.	Fat Acidity[1]	Nonreducing Sugars[2]	Germin. (%)	CO_2 (%)	O_2 (%)	Mold Count (*per g.*)
95% Relative humidity. Initial moisture: 27.0%; final moisture: 25.3–27.4%.							
0	1	22.0	141.5	82.0	0.01	20.95	55,000
	2	21.0	139.1	85.5	0.01	20.95	24,000
2	1	20.5	124.6	75.0	—	—	120,000
	2	20.9	122.5	68.0	—	—	525,000
4	1	20.1	117.2	63.5	25.27	0.45	495,000
	2	19.2	109.0	64.5	26.15	0.30	56,000
6	1	23.5	99.7	47.0	26.17	0.48	115,000
	2	25.9	102.0	44.0	26.36	0.31	134,000
8	1	22.8	95.4	37.0	27.04	0.46	650,000
	2	27.2	89.7	42.0	26.92	0.28	70,500
10	1	27.5	78.7	38.5	27.00	0.48	415,000
	2	24.9	80.0	43.0	27.04	0.31	103,000
12	1	27.4	67.5	0.0	27.12	0.45	13,500
	2	27.5	75.7	23.5	27.27	0.45	77,500
100% Relative humidity. Initial moisture: 30.2%; final moisture: 27.7–30.2%.							
0	1	23.7	126.4	71.0	0.01	20.95	170,000
	2	26.5	150.2	78.0	0.01	20.95	39,000
2	1	20.5	108.5	62.5	—	—	275,000
	2	22.7	128.9	66.5	—	—	60,000
4	1	22.6	78.7	32.0	33.79	0.41	525,000
	2	21.8	88.6	37.0	35.20	0.00	275,000
6	1	22.2	59.4	10.0	36.34	0.00	1,050,000
	2	23.9	66.2	14.0	36.62	0.00	425,000
8	1	27.3	45.6	2.0	36.35	0.00	700,000
	2	26.8	48.2	0.0	37.03	0.00	1,500,000
10	1	24.3	20.7	0.0	31.67	1.03	2,200,000
	2	29.2	21.2	0.0	33.07	0.31	2,000,000
12	1	23.9	18.4	0.0	29.70	0.31	1,450,000
	2	22.5	20.6	0.0	30.13	0.37	2,500,000

[1] Expressed as mg. of potassium hydroxide per 100 g. corn, dry basis.
[2] Expressed as mg. of sucrose per 10 g. corn, dry basis.

the initial mold count of the corn was lower and there was a less rapid increase in fat acidity and a slower decrease in nonreducing sugars and viability.

The influence of moisture and time on the predominant molds is of particular interest. In the corn stored at 18–20% moisture (80%

relative humidity), *Penicillium* sp., predominated initially, but as storage continued, *A. glaucus* (trial 1) or *A. flavus* (trial 2) was present in the greatest numbers. When the samples were stored at about 24% moisture (90% relative humidity), *A. glaucus* was, in general, the most abundant species present. At 27 to 28% moisture (95% relative humidity) this mold was proportionally less abundant and *A. candidus* and *A. flavus* became more important. At 31–32% moisture (100% relative humidity), *A. glaucus* and *A. candidus* were virtually absent, and *A. flavus* and *A. tamarii* (together with *Fusarium* sp. for test 2), were the most prominent species at the end of the trial.

Although an increase in mold count was usually associated with an increase in fat acidity and a decrease in nonreducing sugars, there were several instances where these relations did not hold, particularly with fat acidity. For example, in the storage trials at 100% relative humidity (about 31% moisture), the mold count increased more rapidly in the first trial than in the second, and this was associated with a much more rapid increase in fat acidity and loss of nonreducing sugars. The highest fat acidity was found on the fourth day of the first trial and on the eighth day of the second, while the mold counts continued to increase to the end of both trials. The increases in mold count were accompanied by progressive decreases in nonreducing sugars throughout the storage period in both trials. In these tests fat acidity seemed to be a relatively poor measure of the extent of spoilage of the seed; the quantity of nonreducing sugars was a better measure. These observations are in agreement with the results of an earlier study (2).

The anomalies in the relation between mold count and fat acidity appeared to be associated with differences in the microflora which predominate. Thus, fat acidity increased rapidly when *A. candidus*, *A. flavus*, *Penicillium* sp., or *Fusarium* sp. predominated, whereas *A. glaucus*, while associated with a decrease in nonreducing sugars, appeared to have less influence on fat acidity than the first-mentioned molds.

Nonaerated Samples. In comparison with the aerated samples there was relatively little change in mold count, fat acidity, and nonreducing sugar content for the nonaerated samples during the 12-day storage period. At the higher moisture levels where the most pronounced changes occurred there was almost no change in fat acidity, a significant decrease in nonreducing sugars, and erratic fluctuations in the numbers and kinds of molds.

Of particular significance is the increase of mold populations at 100% relative humidity, where the carbon dioxide concentration of the interseed atmosphere was in the order of 30 to 35% and the oxygen concentration was 0 to 1% after the first few days of the trial. The

present studies indicate that some molds, especially *Cephalosporium* sp., continued to grow in an atmosphere of low oxygen tension approaching anaerobic conditions.

Discussion

These experiments clearly show that not only mold count but also the kinds of molds present, the length of time, and the conditions under which they have been growing on moist corn influence the amounts of fatty acids and nonreducing sugars which are present. The decreases in nonreducing sugar values are a more accurate measure of the deterioration which occurs than are increases in fat acidity but neither of these biochemical tests alone provides accurate information on the extent of damage to the grain. A combination of the two, especially with identification of the molds present, gives a more complete picture than either alone. The prevalence of different species of molds with the time and conditions of storage illustrates the complex ecologic succession encountered where a variety of organisms are competing in a dynamic, fluctuating, biological environment. The metabolic processes of such a mixture of mold species may be further complicated by bacterial growth at the highest moisture level employed in these trials.

The increase in mold count at low oxygen tensions confirms the authors' previous findings with corn that nearly anaerobic conditons (about 0.1% oxygen), although greatly reducing mold development, failed to prevent the growth of all species with consequent deterioration of the grain (2). These observations indicate that the spoilage of high-moisture grain cannot be completely prevented by storing it under hermetically-sealed conditions. It is known that various fungi differ in their oxygen requirements. The present study shows that some molds are able to grow at very low oxygen tensions. Studies with pure cultures of individual species of molds, growing on sterile grain are needed to establish their minimal oxygen requirements and their relative influence on the fat acidity and nonreducing sugar content of the grain.

Acknowledgments

The authors are indebted to J. Goodman for several of the mold counts, and to Ting Chien for determining the moisture contents of the air-dried samples.

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FACTORS AFFECTING THE COLOR OF MACARONI. IV. SEMOLINA PARTICLE SIZE [1]

G. N. IRVINE and J. A. ANDERSON

ABSTRACT

Rates of enzymic destruction of pigment during mixing and macaroni color were determined for three series of semolina particle-size fractions. For the first series, a sample of durum wheat was experimentally milled to obtain a wide range of particle sizes; the second series was obtained by fractionating a commercial semolina on a Ro-Tap; the third series was obtained by reducing and again fractionating a single fraction of the commercial semolina which was retained by a 60- and passed through a 40-mesh sieve. In general, enzymic destruction of pigment increased with decreasing particle size. Macaroni color, on the other hand, passed through a marked optimum with the first two series, the fraction passing through 40- and held on 60-mesh being decidedly superior in color. The principal degrading factors for the finer size fractions were lower yellow color and dullness, which appears to be partly a function of size alone and partly due to an increasing brownness.

During the course of a kinetic study of the lipoxidase oxidation of the carotenoid pigments in macaroni doughs, it was observed (1) that grinding semolina to flour increased the amount of pigment destroyed during the initial phase of the oxidation reaction. The present study was undertaken with two objectives in view: to investigate the lipoxidase activity of the various semolina particle-size fractions making up semolina, and to obtain data on the macaroni-making quality of these particle-size fractions since none are available in the literature.

In 1946 Morris, Alexander and Pascoe (4) showed that protein and ash were differentially distributed in the wheat kernel, being higher in the outer endosperm area than in the center. Wichser, Shellenberger and Pence (5) used these results in interpreting their data on the physical properties of flour fractions separated with a Roller particle-size analyzer. They found that protein and ash were highest in the smallest particle-size fractions and concluded that the largest particles had come from the central endosperm core which, they suggest, is hardest and thus has the least tendency to break up during the milling process. The hardness of the core, in spite of its lower protein con-

[1] Manuscript received September 10, 1951. Presented at the Annual Meeting of the American Association of Cereal Chemists in Minneapolis, May 1951. Paper No. 114 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, Manitoba, and No. 292 of the Associate Committee on Grain Research.

tent, probably results from failure of the core to obtain as much water during tempering as the outer portions of the kernel.

Miller and Kummerow (3) investigated the lipoxidase activity of flour and flour products and were the first to report this activity in flour. They observed that the activity increased as the grade of flour decreased and that bran and defatted germ exhibited a much higher activity than did the patent flour fraction.

Winston and Jacobs (6) reported in 1947 that pigment loss during spaghetti processing increased markedly with increasing ash content of the durum flour used; losses as high as 80% were found with a flour of 1.86% ash.

Materials and Methods

Three different series of particle-size fractions have been investigated: the first comprised nine fractions separated on a Ro-Tap sifter from an experimentally milled semolina; the second series comprised six fractions obtained by fractionating a commercial No. 2 semolina; the third comprised five fractions obtained by reducing a single fraction (through 40-mesh, held on 60-mesh) of this commercial semolina and fractionating it.

Fractions separated on the Ro-Tap were classified by size. In all, eight fractions could be obtained using the sieves. One further fraction, the coarsest, was obtained from the experimentally milled semolina by carefully eliminating as much bran as possible from the coarsest tailings from the purifier. The fractions are numbered as follows:

No. 1 hand purified tailings;
2 retained on 30-mesh;
3 thru 30 held on 35;
4 thru 35 held on 40;
5 thru 40 held on 60;
6 thru 60 held on 80;
7 thru 80 held on 100;
8 thru 100 held on 120;
9 thru 120 (flour).

These numbers are used throughout to refer to all three series of fractions.

Protein and ash determinations were made on each of the semolina fractions. For pigment determinations, the fractions coarser than 60-mesh were ground to pass this mesh and an overnight extraction with water-saturated normal butyl alcohol was used for all fractions. This procedure should make the amount of pigment extracted independent of the size of particle in the fraction. Macaroni was proc-

essed from each fraction by the micro method using a constant absorption of 31%. It is well known that absorption requirements increase with decreasing particle size but at this fairly high absorption the differences between the fractions were not sufficiently large to cause concern in interpretation of the macaroni data. Good macaroni samples were obtained from each fraction. Macaroni color was measured with a Bausch and Lomb color comparator; color scores based on these readings are calculated from the relation, C.S. = $Y^2/W(R + B)$. The sum, Red plus Black, is recorded in the tables as Brown. The lipoxidase activity was followed by processing discs at various mixing times (2) and calculating the rates of pigment destruction.

Results and Discussion

The results obtained with the laboratory milled semolina are shown in Table I. The trend in ash values is similar to that noted by Wichser

TABLE I

SEMOLINA AND MACARONI DATA ON LABORATORY MILLED FRACTIONS

Fraction	Semolina				Macaroni			
	Ash	Protein	Pigment	Enzymic Activity	Yellow	Brown	Pigment	Color Score
	%	%	*p.p.m.*	*p.p.m./min.*	%	%	*p.p.m.*	
1	0.56	11.8	3.60	0.22	39.3	43.0	3.02	2.0
2	0.54	11.9	3.58	0.23	39.9	44.0	3.09	2.3
3	0.60	12.1	3.67	0.34	41.0	41.9	2.98	2.4
4	0.60	12.7	3.75	0.34	39.8	41.8	3.06	2.1
5	0.60	13.5	3.86	0.29	47.2	37.6	3.18	3.9
6	0.64	13.5	3.89	0.42	42.5	41.5	2.93	2.7
7	0.65	13.3	3.90	0.57	38.8	41.8	2.84	1.9
8	0.71	13.2	3.91	0.63	33.8	44.4	2.84	1.2
9	0.92	14.2	4.06	0.55	31.6	49.7	3.11	1.1

et al., but the trend in protein values is somewhat different. They noted a continuous increase in protein with decreasing particle size, whereas the present results indicate a slight decrease from fractions 6 to 8 (the same trend also occurred with the commercial semolina, see Table II). The difference between the two investigations probably results from the different systems employed in milling semolina and flour.

Table I shows that pigment content and enzymic activity of the semolina fractions tend to increase with decreasing particle size. Variations in macaroni pigment content are slight owing to the comparatively low enzymic activity of the wheat sample used. There is, however, a wide variation in macaroni color, which is reflected in the

macaroni color scores. Two factors are responsible for the lack of correspondence between yellow pigment and macaroni color: the brown color tends to mask the yellow when it reaches substantial values; and the opacity of the macaroni, which was found to increase markedly with decreasing particle size, reduces severely the intensity of the yellow color for a given pigment concentration. The increase in opacity with decreasing particle size is partly due to the smaller particles yielding somewhat tougher doughs at the fixed absorption. However, the increase in opacity is quite marked by fraction 6, whereas little change has taken place in consistency. The differences in opacity are greatly magnified at longer mixing times. The result of the interaction of these factors is that the color score indicates a pronounced optimum macaroni color for fraction 5. This fraction constitutes the largest single fraction in our laboratory product and also in a commercial grade 2 semolina. Brown color reaches an optimum low for this fraction

TABLE II

SEMOLINA AND MACARONI DATA ON COMMERCIALLY MILLED FRACTIONS

Fraction	Semolina				Macaroni			
	Ash	Protein	Pigment	Enzymic Activity	Yellow	Brown	Pigment	Color Score
	%	%	*p.p.m.*	*p.p.m./min.*	%	%	*p.p.m.*	
4	0.56	10.7	5.11	0.59	42.7	40.8	4.04	2.7
5	0.58	11.1	5.17	0.70	43.1	42.4	3.93	3.0
6	0.62	11.4	5.40	0.92	38.0	45.9	3.54	1.9
7	0.60	11.2	5.33	1.02	36.6	43.7	3.17	1.6
8	0.60	11.0	5.30	1.45	35.0	44.7	3.08	1.4
9	0.85	11.6	5.43	1.28	30.8	50.7	3.29	1.0

while yellow color is correspondingly high. Fraction 5 probably represents the optimum size for removal of aleurone material from the particles and for removal of bran particles by purification. The macaroni from the larger size fractions was more orange in color and somewhat speckier, while that from the smaller sizes was increasingly brownish and opaque.

The results obtained with the commercial semolina are given in Table II. For this series, the ash, protein, and pigment values for the semolina fractions fall off slightly for fractions 7 and 8, probably as a result of the reduction phase of the milling. The flour fraction (No. 9), on the other hand, yields the highest value of each of these factors, as was the case for the previous series. Enzymic activity increases continuously for fractions 4 to 8, then drops for the flour fraction, as it did in the previous series. This behavior appears to be at variance

with the results of Winston and Jacobs, whose work suggests that enzymic activity increases markedly with increasing extraction and ash content of durum flour. The present evidence suggests a similar trend for all but the flour fraction. There is one important difference between the flour fractions used in this study and the flours used by Winston and Jacobs. In our flour fractions, the germ contamination is concentrated in a small fraction of the total endosperm material; in their flours a similar amount of germ, and proportionally more bran, are distributed throughout a much larger bulk of flour. In their flour there is thus a marked dilution of the anti-oxidants, which are concentrated in the germ; at the same time, higher ash concentration suggests increased amounts of peripheral layers of the kernel that are high in enzyme activity.

The macaroni data show similar trends to those obtained with the previous series. Yellow color shows an optimum for fraction 5 al-

TABLE III

SEMOLINA AND MACARONI DATA ON THE SUB-SERIES FROM FRACTION NO. 5 OF THE COMMERCIALLY MILLED SEMOLINA

Fraction	Semolina				Macaroni			
	Ash	Protein	Pigment	Enzymic Activity	Yellow	Brown	Pigment	Color Score
	%	%	*p.p.m.*	*p.p.m./min.*	%	%	*p.p.m.*	
5	0.57	11.2	4.94	0.76	45.0	35.9	3.67	3.0
6	0.51	11.0	4.91	0.70	48.5	33.0	3.66	3.9
7	0.45	11.0	4.86	0.78	48.9	32.2	3.70	3.7
8	0.46	10.7	4.90	0.89	45.2	30.9	3.51	2.8
9	0.63	11.1	5.06	1.09	40.1	37.1	3.49	1.9

though brown color increases continuously from fractions 4 to 9. Pigment content decreases markedly and is more closely reflected by the yellow color.

Data for the sub-series obtained by reducing the No. 5 fraction of the commercial series are given in Table III. For this series, the trend in ash, protein, and pigment values suggests the effect of reduction on the commercial No. 5 fraction. Apparently some higher protein and ash material is removed, yielding sub-fraction 6, with lesser amounts of more heavily scalped material yielding fractions 7 and 8. Fraction 9 appears to be the only one which contains any appreciable amount of the higher protein and ash material removed from the larger particles. Enzymic activity is highest for the flour fraction (No. 9), whereas the activity of both the commercial and laboratory milled flour fractions was lower than that of the immediately preceding

fraction. This behavior tends to support the view that the decrease in activity of the previous two flour fractions results from the inclusion of anti-oxidant material from the germ.

The macaroni data markedly reflect the "cleaning up" effect of reduction; fractions 6 and 7 yield macaroni superior to the best of the original fractions separated. Macaroni pigment content falls off for fractions 8 and 9 owing to the greater enzymic activity of these fractions. The brown color improves from fractions 5 to 8 and rises sharply for the flour fraction. This results in higher values of yellow for fractions 6 and 7.

Data for all three series of fractions suggest that enzymic activity increases as particle size decreases; moreover, the last sub-series indicate that this effect is partially due to particle size alone. However, in a normal semolina the greatest effect is due to the enzyme-rich material which is present in the finer fractions.

There appear to be three major factors which interact to produce an optimum particle size fraction for macaroni making:

(1) Optimum efficiency of bran removal which occurs at the level of fraction 5, i.e., material passing through a 40- and over a 60-mesh sieve.
(2) Opacity of macaroni, which increases with decreasing particle size.
(3) Enzymic activity of semolina, which also increases with decreasing particle size.

Although coarser material does not yield macaroni of quite as good color as the number five fraction, the differences are probably largely offset, for making macaroni long goods, by the lower absorption required for processing.

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HYGROSCOPIC EQUILIBRIUM OF BROWN RICE [1,2]

DAVID F. HOUSTON

ABSTRACT

Equilibrium moisture contents of Caloro brown rice at 25°C. (77°F.) have been determined for atmospheric relative humidities of 11 to 93%, together with the rates of approach to equilibrium. Moisture contents at 10% R.H. increments, beginning at 10%, are: 6.2, 8.0, 9.5, 10.9, 12.3, 13.5, 14.8, 16.2, and 19.1%.

The extent of hysteresis effects in sorption of moisture by the brown rice is indicated by the fact that rice brought to equilibrium at 40 to 65% relative humidity from a dry condition attains a moisture content 0.7 to 1.0% lower than that resulting from direct drying of moist rice to the same relative humidity.

Maintenance of the quality of brown rice during storage is affected to a great extent by the moisture content of the grain (5). Because this will vary with atmospheric humidity conditions, it is desirable to know what the equilibrium moisture content of brown rice is with regard to the relative humidity of the storage atmosphere. Previous investigations (3, 6) of these relations for rice and rice by-products have omitted brown rice; hence a study has been made to add this information.

A hysteresis effect is shown in the adsorption and desorption of moisture by rice (10, 12) as well as by cereals in general (2, 8). Several measurements of this effect on brown rice have been made and are included in this report.

Materials and Methods

The test material was rubber-roll-shelled Caloro brown rice from the 1950 harvest, and contained 14.8% moisture as determined by the A.O.A.C. vacuum-oven method (1). All moisture values are on the wet basis.

The method of establishing moisture equilibrium was that of Evans and Critchfield (4), the adequacy of which has been discussed by Kline (7). A sub-sample of approximately 5 g. of rice was suspended in a copper-screen basket (1 in. diameter, 0.75 in. high) on a wire through narrow glass tubing in the stopper of a 300-ml. wide-mouth bottle

[1] Manuscript received August 13, 1951.

[2] Contribution from Western Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture, Albany, California. Report of a study made under the Research and Marketing Act of 1946.

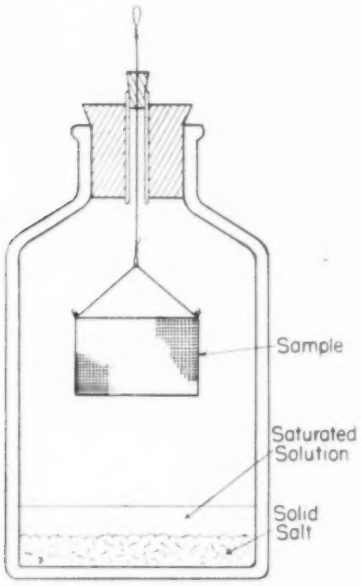

FIG. 1. Apparatus used in determination of moisture equilibria.

(Fig. 1). The glass tube was closed between weighings by a paraffined cork through which the wire passed. A sample was weighed without removal from the conditioning atmosphere by supporting the bottle on a bridge over the balance pan and suspending the wire from the balance hook. The desired relative humidities were achieved by placing saturated salt solutions and excess solid salt in the bottom of the bottles (Table I).

TABLE I

RELATIVE HUMIDITIES OVER SATURATED SALT SOLUTIONS AT 25°C. (77°F.)

Salt[1,2]	*Relative Humidity* %
Lithium chloride	11.1
Potassium acetate	22.5
Magnesium chloride hexahydrate	32.5
Potassium carbonate	43.7
Sodium dichromate dihydrate	53.3
Sodium nitrate	64.4
Sodium chloride	75.4
Potassium chloride	84.3
Ammonium dihydrogen phosphate	92.5

[1] Taken from data of Wink and Sears (13) except for potassium chloride, which is from data of Stokes and Robinson (11).
[2] Distilled water was used for 100% relative humidity.

The samples were stored in a constant-temperature room at 25°C. (77°F.) and weighed periodically until they reached constant weight or changed by an amount equivalent to less than 0.05% moisture in a week. Equilibrium moisture contents were calculated from the original moisture and the weight change.

Estimation of hysteresis effects was made by placing samples which had reached equilibrium at low relative humidities into chambers of higher relative humidity, again determining equilibrium moisture, and comparing the values with those obtained by approach from the more moist condition.

Results and Discussion

Rates of approach to moisture equilibrium at the different relative humidities are shown in Fig. 2. The results are, of course, dependent

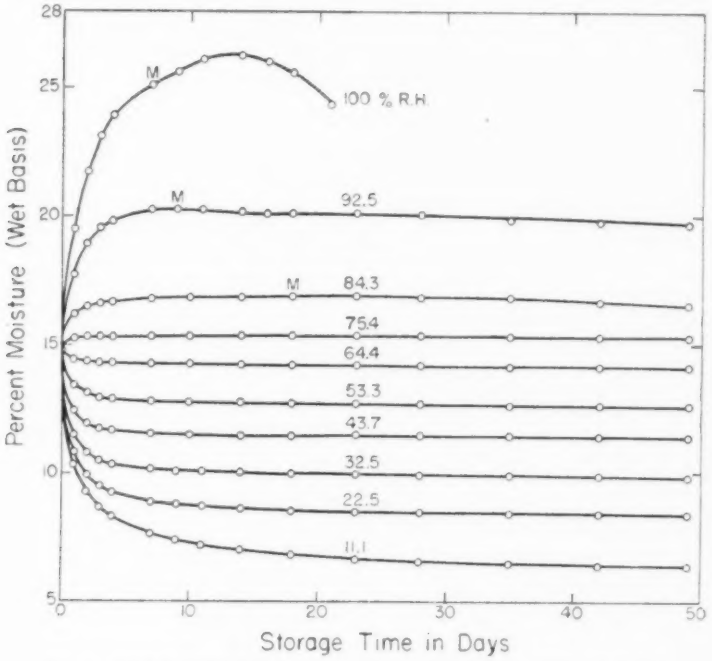

FIG. 2. Rates of approach to equilibrium in brown rice at 25°C. (77°F.) at various relative humidities. M indicates time at which sample was visibly moldy.

on the accuracy with which the original moisture determination measures the actual moisture content of the grain. Changes in weight of basket assemblies were negligible. The work of Oxley (9) on wheat indicates that the 5-g. sample is sufficiently large to avoid significant

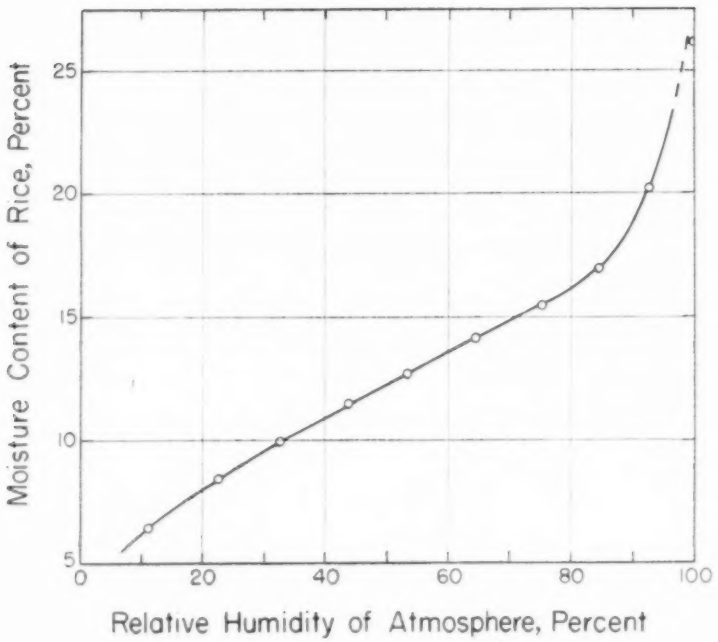

FIG. 3. Relation of equilibrium moisture content of brown rice to atmospheric relative humidity at 25°C. (77°F.).

sampling error. Equilibrium values plotted in Fig. 3 against relative humidity show the usual sigmoid curve obtained for many biological materials.

Action of microorganisms became evident at relative humidities of 84% and above, which caused increased interference with establish-

TABLE II

COMPARISON OF BROWN RICE EQUILIBRIUM MOISTURE CONTENTS AT VARIOUS RELATIVE HUMIDITIES WITH REPORTED VALUES FOR POLISHED AND ROUGH RICE

Sample Description	Per Cent Relative Humidity of Atmosphere[4]								
	10	20	30	40	50	60	70	80	90
Brown rice[1]	6.2	8.0	9.5	10.9	12.3	13.5	14.8	16.2	19.1
Polished rice[2]	5.2	7.6	9.2	10.5	12.0	13.4	14.8	16.4	18.8
Rough rice[2]	4.4	6.5	7.9	9.1	10.4	11.8	13.2	14.8	17.6
Rough rice[3]	—	7.6	9.0	10.2	11.3	12.6	13.8	15.3	18.1

[1] Present investigation on Caloro (short-grain) rice.
[2] Data of Karon and Adams (6) for field-dried Rexoro (long-grain) rice.
[3] Data of Coleman and Fellows (3) converted to wet basis (variety not given).
[4] All at 25°C. (77°F.).

ment of equilibrium as the humidity increased. At 100% relative humidity the sample began to show considerable weight losses before equilibrium was reached, and the actual value is undoubtedly higher than that shown in Fig. 3. Mold growth was found at 75% relative humidity after three and one-half months of storage.

The equilibrium moisture values agree well with those of Karon and Adams (6) for long-grain white rice (Table II), although some divergence becomes apparent at the lowest relative humidities. Somewhat lesser agreement is found between brown and rough rice. The rough rice shows lower moistures in concordance with the lower moisture contents of hulls (6).

Equilibrium moisture contents are dependent to some extent, because of hysteresis effects, on the direction from which they are approached. This has been previously noted for rice (10, 12) as well as for other grains (2, 8). Measurements to determine the possible magnitude of this effect show (Table III) that in the range of 40 to 65% relative humidity the moisture content reached by adsorption is nearly 1% lower than that following desorption at the same relative humidity. However, if dry rice is moistened and then brought to equilibrium by final desorption, the moisture content is nearly the same as that reached by drying an originally moist rice. Samples of rice brought to a single moisture content by drying in one case and by moistening in another will be in equilibrium with atmospheres of different relative humidities. Hence, caution should be used in comparing the results of any experiments involving naturally moist rice and rice which has been dried and remoistened.

TABLE III

Effect of Direction of Approach on Equilibrium Moisture Contents of Brown Rice at 25°C. (77°F.)

Moisture Content Before Equilibration	Relative Humidity, %		
	43.7	53.3	64.4
	Equilibrium Moisture[2]		
%	%	%	%
14.8	11.4	12.7	14.2
6.4	10.5	11.7	13.2
8.4	—	11.8	13.3
9.9	—	—	13.4
13.4[1]	11.4	—	—

[1] This sample had previously been equilibrated at 9.9% moisture, and then at 13.4%.
[2] Measurements were continued in all cases until the sample showed a change of less than 0.05% moisture in a week.

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VARIATIONS IN THE PROTEIN AND AMINO ACID CONTENTS OF DIFFERENT OAT VARIETIES [1]

KENNETH J. FREY [2]

ABSTRACT

The average protein content of the grain of 14 oat varieties was 25% higher in 1949 than in 1947. Although the contents of lysine, leucine, and methionine were 47, 47, and 43% higher, respectively, in 1949 than in 1947, the tryptophan content was only 12% higher. These data show that environmental variation associated with season and planting area tend to change the proportions of certain amino acids in the oat protein. Such changes in the tryptophan component were not as apparent in Mindo and Andrew varieties as in Mohawk, Clinton, and Huron.

The average percentages of tryptophan, lysine, leucine, and methionine in the oat protein were 1.2, 3.4, 7.0, and 1.2, respectively.

The protein of oats is generally considered to be poor in nutritional quality. It has been suggested that this poor quality may be due to a deficiency of one or more of the essential amino acids, usually tryptophan. With this in mind, a study of the oat varieties grown at the Michigan Agricultural Experiment Station was conducted to determine: first, how much variation in per cent of protein, tryptophan, lysine, leucine, and methionine existed among the varieties, and second, how uniform they were in their contents of these five components from year to year.

Materials and Methods

The 14 oat varieties used in this study (Table I) were grown in yield tests at East Lansing, Michigan in 1947 and 1949. The yield tests were located on different soil areas in the two years, but the fertilizer treatments were the same. The seed of each variety from two replications was composited, thoroughly mixed, and 100-g. portions were selected by using a grain divider. These samples were air dried, ground to pass a 40-mesh sieve and analyzed for protein, tryptophan, lysine, leucine, and methionine.

Protein nitrogen was determined by the Kjeldahl-Wilcarth-Gunning method and converted to protein by use of the factor 6.25.

Tryptophan analyses employing the method of Schweigert *et al.*

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[2] Assistant Professor of Farm Crops, Michigan Agricultural Experiment Station, East Lansing, Michigan.

(6), were preceded by hydrolysis of the oat samples with pepsin and trypsin according to the procedure of Wooley and Sebrell (7).

The oat samples were hydrolyzed for the lysine, leucine, and methionine determinations by treating the ground material with 6 *N* hydrochloric acid for eight hours at 15 lbs. pressure, after which the acid was neutralized with sodium hydroxide. Lysine, leucine, and methionine in the hydrolysates were evaluated by the methods of

TABLE I

THE PERCENTAGES OF PROTEIN, TRYPTOPHAN, LYSINE, LEUCINE, AND METHIONINE IN FOURTEEN OAT VARIETIES GROWN IN 1947 AND 1949 AT EAST LANSING, MICHIGAN

	Protein		Tryptophan		Lysine		Leucine		Methionine	
	1947	1949	1947	1949	1947	1949	1947	1949	1947	1949
	%	%	%	%	%	%	%	%	%	%
Andrew	12.8	14.8	0.15	0.17	0.37	0.52	0.82	1.09	0.17	0.19
Beaver	12.6	16.6	0.16	0.18	0.36	0.59	0.74	1.19	0.15	0.23
Bonda	13.4	16.6	0.17	0.18	0.40	0.59	0.83	1.25	0.14	0.22
Clinton	11.7	15.9	0.15	0.19	0.36	0.61	0.75	1.19	0.14	0.21
Colo	10.7	16.0	0.14	0.19	0.36	0.55	0.70	1.14	0.13	0.22
Eaton	13.4	15.4	0.16	0.17	0.42	0.55	0.89	1.17	0.15	0.20
Huron	9.3	13.8	0.13	0.15	0.29	0.49	0.61	1.01	0.12	0.18
Mindo	11.7	15.0	0.15	0.18	0.34	0.55	0.74	1.16	0.13	0.21
Mohawk	11.9	15.3	0.15	0.17	0.39	0.54	0.78	1.09	0.14	0.20
Vicland	12.4	15.3	0.17	0.18	0.39	0.53	0.82	1.07	0.14	0.17
Wolverine	11.9	15.9	0.14	0.17	0.36	0.55	0.73	1.14	0.14	0.20
Worthy	11.2	14.2	0.14	0.16	0.38	0.53	0.73	1.06	0.14	0.19
C.I. 3656	14.5	16.0	0.16	0.17	0.44	0.62	0.93	1.29	0.17	0.23
C.I. 5298	15.8	16.8	0.19	0.18	0.47	0.63	1.03	1.32	0.18	0.21
Mean	12.4	15.5	0.16	0.17	0.38	0.56	0.79	1.16	1.43	0.20

Analysis of Variance
Mean Square

Source	D.F.	Protein	Tryptophan	Lysine	Leucine	Methionine
Year	1	70.10[2]	0.00251[2]	0.2268[2]	0.9181[2]	0.0257[2]
Variety	13	2.65[1]	0.00028	0.0030[2]	0.0165[2]	0.0004
Error	13	0.73	0.00012	0.00060	0.0026	0.0002

[1] Exceeds the 5% level of significance.
[2] Exceeds the 1% level of significance.

McMahan and Snell (4), Schweigert *et al.* (5), and Lyman *et al.* (3), respectively.

Since the material from 1947 and 1949 was analyzed at different times, and since microbiological methods are known to give varying results from one assay to another, Huron variety from 1947 was included with the 1949 material as a check. In all assays the check was within 9% of the original values obtained in 1947, so it seemed safe to

compare the amino acid percentages from the two years directly with one another.

All data presented herein are expressed as a percentage of the air-dried samples. The moisture percentages of the samples averaged 6% and varied so little that correction to an oven-dried basis was unnecessary.

Results and Discussion

The percentages of protein, tryptophan, lysine, leucine, and methionine for the 14 oat verieties are given in Table I. Huron variety and C.I. 5298 represented the extremes in low and high protein percentages, respectively, in both years. The range in protein percentages was 6.5 in 1947 and 3.0 in 1949. Likewise, Huron and C.I. 5298 contained the lowest and highest percentages of lysine and leucine. Lysine percentage range from 0.29 to 0.47 in 1947 and from 0.49 to 0.63 in 1949, and leucine percentage ranged from 0.61 to 1.03 and from 1.01 to 1.32 in 1947 and 1949, respectively. These lysine value are,

TABLE II

THE PROTEIN, TRYPTOPHAN, LYSINE, LEUCINE, AND METHIONINE CONTENT OF THE SAMPLES GROWN IN 1949 AS A PERCENTAGE OF THE 1947 VALUES

Component	%
Protein	125
Tryptophan	112
Lysine	147
Leucine	147
Methionine	143

in general, lower than the 0.6% lysine in oats reported by Baumgarten *et al.* (1), while the leucine values are higher than their value of 0.7% leucine in oats. The tryptophan content ranged from 0.125% in Huron variety to 0.193% in C.I. 5298 and from 0.153% in Huron to 0.192 in Colo in the two years. The range in methionine percentage was from 0.120 in Huron variety to 0.181 in C.I. 5298 in 1947 and from 0.166 in Vicland to 0.228 in Beaver in 1949.

The average content of protein and each of the amino acids was significantly higher in 1949 than in 1947 as is shown by the analyses of variance at the bottom of Table 1. However, closer scrutiny of the data reveals that protein and tryptophan did not increase in the same proportion as did lysine, leucine, and methionine. This is shown more clearly in Table II where the 1949 values are expressed as percentages of the 1947 values. The average total protein content was increased from 12.4 to 15.5% or about one-fourth while the average tryptophan content was increased from 0.155 to 0.173 or only 12%. Even though in every variety the relative ratio of increase in protein

content was greater than in tryptophan content, there was a great deal of variation among the varieties with respect to the magnitude of the difference between the relative rates of increase of protein and tryptophan. For instance, the protein and tryptophan contents of Mindo variety were 28 and 23% higher, respectively, in 1949 than in 1947, while in Mohawk the increase was 29 and 8%, respectively. In other words, the relative rates of increase of protein and tryptophan were nearly the same in Mindo variety, while in Mohawk the protein increased at a greater relative rate than did tryptophan. Andrew variety reacted like Mindo, and Clinton and Huron varieties behaved like Mohawk.

The average contents of lysine, leucine, and methionine were 47, 47, and 43% higher, respectively, in the 1949 crop than in the 1947 crop, showing that these amino acids reacted differently than tryptophan did. Although the basic reasons for these variations are not

TABLE III

CORRELATION COEFFICIENTS OF THE 1947 PERCENTAGES OF PROTEIN TRYPTOPHAN, LYSINE, LEUCINE, AND METHIONINE IN FOURTEEN OAT VARIETIES WITH PERCENTAGES OF THE SAME COMPONENTS IN 1949

Item	*Correlation Coefficient*
Protein 1947 and 1949	0.67[1]
Tryptophan 1947 and 1949	0.43
Lysine 1947 and 1949	0.66[1]
Leucine 1947 and 1949	0.74[1]
Methionine 1947 and 1949	0.29

[1] Exceeds the 1% level of significance.

known, it is clearly evident that there was a different balance of certain amino acids in the protein of a majority of the oat varieties in 1949 than in 1947.

The fact that the amino acid balance was shifted within the oat protein by growing the oat varieties under a different set of environmental conditions leads to the speculation that it might be possible to change the proportion of certain limiting amino acids in the oat protein in a desired direction by changing the conditions under which the oats are grown. Examples of growing conditions which might be controlled are tillage practices and fertilizer application.

The data also suggest that certain varieties of oats do not change as much in amino acid balance as others when grown under different environmental conditions. Mindo and Andrew varieties showed almost as much proportional increase in tryptophan as in protein while Mohawk, Clinton, and Huron showed a much greater relative increase in protein than in tryptophan. This fact would be significant if the

suggestion of Frey (2) for improving oats as a source of protein were followed: namely, that oats could be improved as a protein source simply by selecting for high nitrogen percentage. It now appears that this method of selection might work for certain varieties or strains but not for others.

For all of the oat samples lysine and leucine made up 3.4 and 7.0% of the protein, respectively, and tryptophan and methionine each made up 1.2%.

Table III shows that the percentages of protein, leucine, and lysine in 1947 were significantly correlated with those in 1949. On the basis of these correlations it seems safe to conclude, at least for the varieties used in this study, that the oat varieties which have a high percentage of protein, leucine, or lysine when grown under one set of environmental conditions, will tend to remain high relative to other varieties when grown in a different environment. One noticeable exception to this was protein percentage in Colo variety. It increased from one of the lowest protein varieties in 1947 to one of the highest in 1949. Neither tryptophan nor methionine percentages for the two years was correlated significantly.

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RELATIVE STABILITY OF THIAMINE MONONITRATE AND THIAMINE CHLORIDE HYDROCHLORIDE IN ENRICHED FLOUR [1]

C. M. Hollenbeck and H. G. Obermeyer [2]

ABSTRACT

Storage tests were conducted on samples of flour enriched with thiamine mononitrate and thiamine chloride hydrochloride, respectively, under both normal and accelerated conditions. The relative stability of thiamine in enriched flour decreased with increased temperature and flour moisture during storage. However, the stability of thiamine mononitrate in flour was influenced less by the temperature and the moisture conditions of the flour than was the stability of thiamine chloride hydrochloride. For example, enriched flour samples with 14.5% moisture lost 40% and 27%, respectively in thiamine chloride hydrochloride but only 5% or less in thiamine mononitrate during storage for 4 months at 38°C. and room temperature.

The increased stability of thiamine mononitrate in enriched flour is attributed to its relatively low hygroscopicity compared to thiamine chloride hydrochloride.

With the advent of the flour enrichment program, several laboratories studied the stability of the enriching vitamins in flour. It was generally recognized that thiamine was slowly destroyed during the storage of enriched flour, and that the rate of destruction was dependent upon the temperature and moisture content of the flour. Early estimates by the Technical Advisory Committee of the Miller's National Federation place the average loss in thiamine during six months dry storage of enriched flour at 5%. Later estimates tended to place the six months dry storage loss at nearer 15%.

In 1945, a report from the laboratories of Anheuser-Busch, Inc., (1) showed losses of 24% and 46%, respectively, in thiamine chloride hydrochloride during storage of enriched flour samples for six months at 72°F. and 100°F. Although these storage losses were observed for thiamine in samples of flour stored in closed, glass bottles, they were indicative of the losses which could occur during regular commerical flour usage, including storage and transportation.

Federal and state enrichment standards (3) for enriched flour specify that it contain not less than 2.0 mg. and not more than 2.5 mg. of thiamine per pound of flour. In the production of enriched

[1] Manuscript received July 27, 1951. Contribution from the Research Laboratories of Merck and Co., Inc., Rahway, N. J. Presented at the Annual Meeting May, 1951.

[2] Present address, General Mills, Inc., Minneapolis, Minnesota.

flour, the losses in thiamine encountered during storage and transportation of the product must be taken into account, since the product must meet the specifications of these standards even after prolonged storage and transportation in hot, humid weather. It is general practice to add an extra amount of thiamine to flour to compensate for any losses that may occur during storage and transportation. In general, a small overage in thiamine is ample, since the losses in thiamine are small during the oridinary handling of flour. However, there is some question as to whether the total allowance for thiamine between the maximum and the minimum in the flour standards (0.5 mg. per lb.) would make up for the losses occurring during handling and storage of enriched flour under unusual conditions.

In a study aimed at increasing the stability of thiamine and thereby eliminating the storage losses in flour, Obermeyer and Schoeffel (4) prepared and tested many different salts of thiamine. These investigators found that the mononitrate salt of thiamine was unusually stable during storage in flour mixtures.

The present report summarizes some of the results of studies on the relative stability of thiamine mononitrate and thiamine chloride hydrochloride in flour, and illustrates the influence of temperature and moisture on the stability of thiamine in flour.

Materials and Methods

Three separate storage tests were conducted on various samples of flour enriched with thiamine chloride hydrochloride and thiamine mononitrate, respectively. In the first test, 1 lb. samples representing nine different commercial flours were enriched in the laboratory and stored in paper bags at room temperature for approximately one year. In the second test, 5 lb. samples of commercially enriched flours were stored in cloth bags for about seven months under room conditions. After about 3 months portions of these commercially enriched samples were also stored in a desiccator at about 70% relative humidity to compensate for the excessive moisture loss that occurred under room humidity. In the third test, flour samples were conditioned to the desired moisture levels by storing in desiccators under controlled humidity, enriched, and stored for four months in sealed glass containers.

The thiamine chloride hydrochloride and thiamine mononitrate used in these studies were U.S.P. grade or its equivalent. In all cases the thiamine was blended with riboflavin, niacin, and iron according to the regular flour enrichment mixture formula prior to the addition to flour. The enrichment mixtures were added to flour samples at levels to give 2.0 to 2.5 mg. of thiamine per pound of flour.

Thiamine and flour moisture determinations were conducted by the official (2) procedures at periodic intervals during the storage tests.

Results

The average rates of loss in thiamine for the flour samples enriched in the laboratory with thiamine chloride hydrochloride and thiamine mononitrate respectively and stored for about one year are shown in Fig. 1.

The average flour moisture at the start of the tests was 12% but during the storage it decreased to 9.2%. The storage temperature

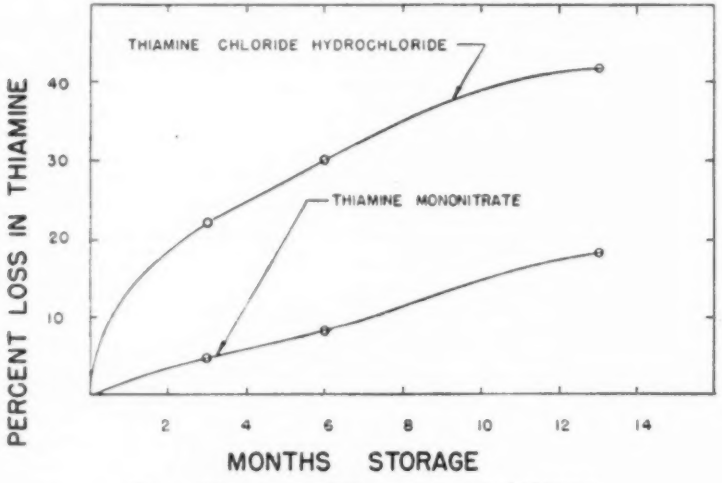


FIG. 1. Relative loss in thiamine during storage of laboratory enriched samples of flour under room conditions.

ranged from a low of 60°F, to a high of 100°F. with an average of about 75°F.

The results of this first storage test show that at the end of 13 months storage the flour samples enriched with thiamine mononitrate lost only 18% in thiamine compared to a loss of 42% in thiamine for the samples enriched with thiamine chloride hydrochloride.

The average rates of thiamine loss for the commercially enriched flour samples are shown in Fig. 2.

Since the humidity of the room was quite low, the flour samples in this second storage test dried out to less than 10% moisture within a few weeks. However, the portions of each sample which were stored in a desiccator at 70% relative humidity absorbed moisture, and at the end of the test storage the moisture content had increased to 11.7%.

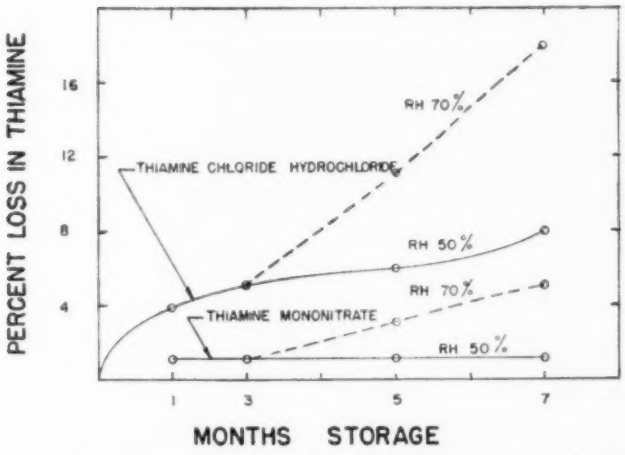


FIG. 2. Relative loss in thiamine during storage of commercial samples of enriched flour at room temperature and 50% and 70% relative humidity.

The curves in Fig. 2 demonstrate the increased rate of loss in thiamine with increased storage humidity (flour moisture). Although thiamine mononitrate is shown to be more stable than thiamine chloride hydrochloride under dry conditions, the difference in the stabilities of the two thiamine salts is even more striking at the higher moisture levels.

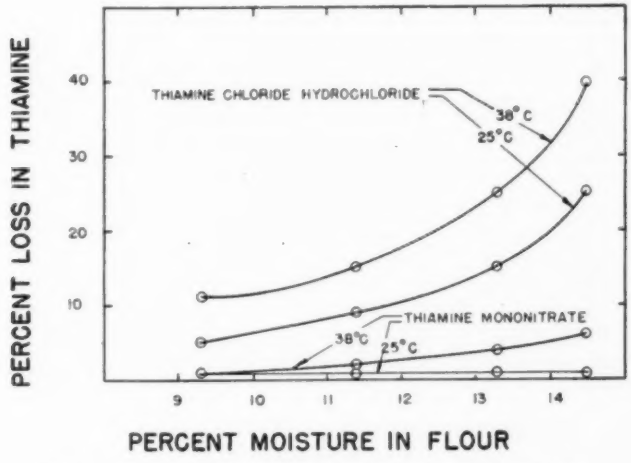


FIG. 3. Effect of flour moisture and storage temperature on the stability of thiamine in flour stored in sealed containers.

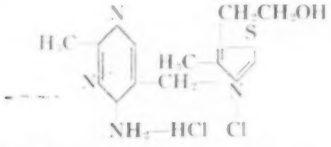
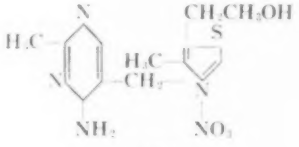
Further illustration of the combined effects of increased temperature and flour moisture on the relative stabilities of the two thiamine salts in enriched flour is shown in Fig. 3.

The results of these storage tests show comparatively small effects of higher temperatures and humidities during flour storage on thiamine mononitrate when compared to the effects of these factors on thiamine chloride hydrochloride. It may be concluded then that flour enriched with thiamine mononitrate is much more likely to withstand adverse storage and transportation conditions and still meet the specifications of enrichment standards than flour enriched with thiamine chloride hydrochloride.

Discussion

Crystalline thiamine chloride hydrochloride is generally found to be stable when stored as a solid for extended periods even at elevated temperatures in the presence of air. It is assumed then, that the decomposition of thiamine in flour is dependent upon its solution in water. This assumption is strengthened by the observation that the rate of decomposition of thiamine in flour is increased by increasing the moisture content of the flour.

A probable explanation of the relative stability of the mononitrate salt of thiamine in flour can be developed from a comparison of the hygroscopicity and water solubility of the mononitrate salt and the hydrochloride salt. Some of the pertinent physical properties of the two salts are as follows:

Name:	Thiamine chloride hydrochloride	Thiamine mononitrate
Formula:		
Molecular weight:	337.3	327.3
Water solubility g./ml.:	1	0.03
pH saturated solution:	2.5–3.0	6.6–7.0
Moisture content % (60% R.H.):	4	0.1

Since thiamine mononitrate is less water soluble and less hygroscopic than thiamine chloride hydrochloride, there is a slower rate of solution of the thiamine in the flour moisture, and consequently a slower rate of decomposition.

In connection with the use of thiamine mononitrate as an enriching agent for flour, the following points might be mentioned:

(1) Thiamine mononitrate is a white crystalline solid, and is fully available and active as vitamin B_1 in nutrition.

(2) It meets all requirements under the federal and state Standards of Identity for enriched flour.

(3) It was made commercially available in January, 1950 and has received wide acceptance by the milling industry.

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COMMUNICATION TO THE EDITOR

Dough Expansion Test

Dear Sir:

Miller *et al.*[1] recently described a small scale dough expansion test.

Several years ago, this laboratory perfected a dough expansion test based on the same principle and the test has since been improved[2,3]. It is derived from the original Pelshenke method which was further developed by using an apparatus that permits measurement of the gas volume as a function of fermentation time. According to our latest findings, an indication of the true baking value of a flour or wheat is given by the product of fermentation time and total gas volume at the time of disintegration of the dough.

E. Maes,
Chief, Central Laboratory,
Ministry of Economic Affairs,
Brussels, Belgium

[1] Miller, H., Edgar, J., and Whiteside, A. G. O. A small scale dough expansion test for the estimation of wheat quality. Cereal Chem. **28**: 188–195 (1951).
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BOOK REVIEWS

Die historische Entwicklung der Mullerei. By Wilhelm Glauer. Second edition, 33 illustrations. Joh. Heinrich Meyer, Braunschweig, Germany, 1951. Price, 3 German marks.

The first edition of this book enjoyed a favorable reception in the English, American, Swedish, Roumanian, Hungarian, and Argentinian technical literature and there is little doubt that this new and much enlarged edition will be equally successful.

The book traces in considerable detail the historical development of the flour milling industry from the mortar and pestle to the most modern pneumatic flour mills. The historical introduction is especially complete and the book contains a large series of excellent illustrations which are explained in the text. The findings of the German Hindukusch expedition of 1935 on the origin of cereals are discussed and it is shown that the plough was on use in Central and Northern Europe at least fifteen hundred years before the Christian era.

The book is well printed and should prove of interest to cereal technologists.

JAKOB LITZENDORFF
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Germany

The Molds and Man: An Introduction to the Fungi. By C. M. Christensen. University of Minnesota Press, Minneapolis, 1951. Price, $4.50.

The purpose of this book as stated by the author is to "introduce the fungi not only to those who expect to go on to more advanced work in mycology but also to those who just want sufficient information about the fungi to be able to talk and think intelligently about them." This purpose has been very well accomplished. After a brief discussion of what fungi are and how they grow, the remainder of the text is given over to the relationship of fungi to their environment. The partnerships and interdependence of fungi with other plants, animals and man are ably and interestingly presented with sufficient wit and philosophic conjecture on the consequences of parasitism and communalism to make the presentation both informative and stimulating.

The fundamental destructiveness of molds and the ingenious use of this characteristic in the development of fermentations and the processing of foods such as cheese, as well as the use of the fungi themselves as comestibles, is interestingly told. The style is informal, often vernacular, but with no tone of "writing down" nor with sacrifice of scientific accuracy. The book concludes with an appendix briefly summarizing the classification of fungi characterizing the chief groups.

JOSEPH C. GILMAN
Iowa State College
Ames, Iowa

Fundamentals of Soil Science. By C. E. Millar and L. M. Turk. Second Ed. 510 pp. John Wiley & Sons, New York, 1951. Price, $5.00.

The second edition represents a very careful and thorough revision of the book. Some portions have been condensed and others elaborated. New chapters on "Processes of Soil Development," "Chemical Properties of Soils and Conserving Soil" have been added. Chapters on "Fruit Soils and Lawn Soils" included in the first have been omitted from the second edition. This represents an increase in chapters from 19 to 20 and pages from 462 to 510. Tabular material is increased by 10 additional tables and new figures by 24.

In revising the volume the authors have incorporated new information, clarified and rearranged subject matter. The new chapter on "Conserving Soil" is a valuable addition and improves the book as a text, as does the inclusion of color plates showing nutrient deficiency symptoms in crops.

The revised edition should find wide use as a text in college courses in elementary soils. It gives a clear, well-arranged and complete treatment of subject matter. The volume will also be of value to others having a technical or practical interest in the soil.

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Cereal Chemistry

EDITORIAL POLICY

Cereal Chemistry publishes scientific papers dealing with raw materials, processes, or products of the cereal industries, or with analytical procedures, technological tests, or fundamental research, related thereto. Papers must be based on original investigations, not previously described elsewhere, which make a definite contribution to existing knowledge.

Cereal Chemistry gives preference to suitable papers presented at the Annual Meeting of the American Association of Cereal Chemists, or submitted directly by members of the Association. When space permits, papers are accepted from other scientists throughout the world.

The papers must be written in English and must be clear, concise, and styled for *Cereal Chemistry*.

Manuscripts for publication should be sent to the Editor in Chief. Advertising rates may be secured from and subscriptions placed with the Managing Editor, University Farm, St. Paul 1, Minnesota. Subscription rates, $11.00 per year. Foreign postage, 50 cents extra. Single copies, $2.50; foreign, $2.60. Back issues, $3.00.

SUGGESTIONS TO AUTHORS

General. Authors will find the last volume of *Cereal Chemistry* a useful guide to acceptable arrangements and styling of papers. "On Writing Scientific Papers for Cereal Chemistry" (*Trans. Am. Assoc. Cereal Chem.* **6**: 1–22. 1948) amplifies the following notes.

Authors should submit two copies of the manuscript, typed double spaced with wide margins on 8½ by 11 inch white paper, and all original drawings or photographs for figures. If possible, one set of photographs of figures should also be submitted. Originals can then be held to prevent damage, and the photographs can be sent to reviewers.

Titles and Footnotes. Titles should be specific, but should be kept short by deleting unnecessary words. The title footnote shows "Manuscript received . . ." and the name and address of the author's institution. Author footnotes, showing position and connections, are desirable although not obligatory.

Abstract. A concise abstract of about 200 words follows title and authors. It should state the principal results and conclusions, and should contain, largely by inference, adequate information on the scope and design of the investigation.

Literature. In general, only recent papers need be listed, and these can often be cited more advantageously throughout the text than in the introduction. Long introductory reviews should be avoided, especially when a recent review in another paper or in a monograph can be cited instead.

References are arranged and numbered in alphabetical order of authors' names and show author, title, journal, volume, first and last pages, and year. The list is given at the end of the paper. Reference numbers must invariably be cited in the text, but authors' names and year may be cited also. Abbreviations for the names of journals follow the list given in *Chemical Abstracts* **40**: I–CCIX. 1946.

Organization. The standard organization involves main sections for abstract, introduction, materials, methods, results, discussion, acknowledgments, and literature cited. Alternately, a group of related studies, each made with different materials or methods, may require a separate section for each study, with subsections for materials and methods, and for results, under each section. Center headings are used for main sections and italicized run-in headings for subsections, and headings should be restricted to these two types only.

Tables. Data should be arranged to facilitate the comparisons readers must make. Tables should be kept small by breaking up large ones if this is feasible. Only about eight columns of tabular matter can be printed across the page. Authors should omit all unessential data such as laboratory numbers, columns of data that show no significant variation, and any data not discussed in the text. A text reference can frequently be substituted for columns containing only a few data. The number of significant figures should be minimized. Box and side heading should be kept short by abbreviating freely; unorthodox abbreviations may be explained in footnotes, but unnecessary footnotes should be avoided. Leader tables without a number, main heading, or ruled lines are often useful for small groups of data.

Tables should be typed on separate pages at the end of the manuscript, and their position should be indicated to the printer by typing "(TABLE I)" in the appropriate place between lines of the text. (Figures are treated in the same way.)

Figures. If possible, all line drawings should be made by a competent draftsman. Traditional layouts should be followed: the horizontal axis should be used for the independent variable; curves should be drawn heaviest, axes or frame intermediate, and the grid lines lightest; and experimental points should be shown. Labels are preferable to legends. Authors should avoid identification in cut-lines to be printed below the figure, especially if symbols are used that cannot readily be set in type.

All drawings should be made about two to three times eventual reduced size with India ink on white paper, tracing linen, or *blue*-lined graph paper; with any other color, the unsightly mass of small grid lines is reproduced in the cut. Lettering should be done with a guide using India ink; and letters should be 1/8 to 1/4th inch high after reduction.

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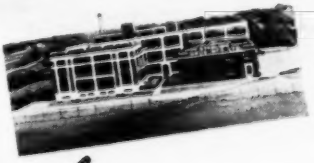

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